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(54) Title: ALZHEIMER'S DISEASE THERAPEUTICS (57) Abstract A method of identifying a therapeutic useful for treating or preventing Alzheimer's disease, which method includes the steps of contacting (a) a first molecule containing the couplone portion of APP (SEQ ID NO: 1) with (b) a second molecule containing the amino acid sequence of G _o (SEQ ID NO: 2) or an APP-associating region of G _o (SEQ ID NOs: 3, 4, or 5), in the presence of a candidate compound; and determining whether the candidate compound interferes with the association of the first and second molecules, such interference being an indication that the candidate compound is a potential Alzheimer's disease therapeutic.		

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ALZHEIMER'S DISEASE THERAPEUTICS

The field of the invention is Alzheimer's disease therapeutics.

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Background of the Invention

Alzheimer's disease (AD) is a progressive degenerative disorder of the brain that afflicts over four million people in the United States. No effective treatment is available. The most characteristic change
10 observed upon post-mortem histopathological analysis of AD-afflicted brain tissue is the presence of neuritic and cerebrovascular plaques containing dense deposits of β -amyloid protein (Selkoe, Cell 58:611-612, 1989). β -amyloid is a 39-43 amino acid peptide (Glennner and Wong,
15 biochem. biophys. Res. Commun. 120:885-890, 1984; Masters et al., Proc. Natl. Acad. Sci. USA 82:4345-4249, 1985) synthesized as part of a larger precursor protein referred to as amyloid precursor protein (APP), which is known to have a number of isoforms in humans (APP₆₉₅, Kang
20 et al., Nature 325:733-736, 1987; APP₇₅₁, Ponte et al., Nature 331:525-527, 1988, and Tanzi et al., Nature 331:528-530, 1988; and APP₇₇₀, Kitaguchi et al., Nature 331:530-532, 1988). The amino terminal of β -amyloid is generated by cleavage of a peptide bond of APP which in
25 APP₆₉₅ lies between Met596 and Asp597.

Although structural alterations of APP are implicated in the pathogenesis of Alzheimer's disease, it remains unknown how they cause the disease. No biological function for APP has been identified, although
30 there is evidence that APP has a receptor-like architecture (Kang et al., Nature 325:733-736, 1987; Ponte et al., Nature 331:525-527, 1988; Tanzi et al., Nature 331:528-530, 1988; Kitaguchi et al., Nature 331:530-532, 1988), is located on the neuronal surface
35 (Dyrks et al., EMBO J. 7:949-957, 1988), and possesses an

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evolutionarily conserved cytoplasmic domain (Yamada et al., Biochem. Biophys. Res. Commun. 149:665-671, 1987).

Summary of the Invention

The methods and therapeutical compositions of the invention are based upon the discovery, described in detail below, that APP forms a complex with G_o , a major GTP-binding protein (or "G protein") in brain. Like all G proteins, a molecule of G_o is made up of one α subunit and one $\beta\gamma$ subunit. Two isoforms of G_o , known as G_{o1} (or G_{oA}) and G_{o2} (or G_{oB}), have been identified; they have slight amino acid differences in their α subunits, and are together referred to herein as G_o . The cDNA sequence and deduced amino acid sequence of the α subunits of each of G_{o1} and G_{o2} (as reported by Strathmann et al., Proc. Natl. Acad. Sci. USA 87:6477-6481, 1990) are shown in Fig. 4a (SEQ ID NO: 2) and Fig. 4b (SEQ ID NO: 28), respectively.

The finding that APP associates with G_o is consistent with related findings concerning other G proteins, as disclosed in a second application (USSN _____) having the same inventor and filing date as the present application, which second application is herein incorporated by reference. The cytoplasmic APP₆₉₅ sequence His⁶⁵⁷-Lys⁶⁷⁶ (SEQ ID NO: 1) possesses a specific G_o -activating function, and is necessary for complex formation of this APP with G_o ; this sequence, sometimes referred to as the "couplone" region of APP, is completely conserved in APP₇₅₁ and APP₇₇₀, as well as in mouse APP₆₉₅. This provides evidence that APP is a receptor coupled to G_o , and suggests that abnormal APP- G_o signalling is involved in the Alzheimer's disease process.

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The invention includes a method of identifying a therapeutic useful for treating or preventing Alzheimer's disease, which method includes the steps of

contacting (a) a first molecule containing the
5 couplone portion of APP (SEQ ID NO: 1) with (b) a second molecule containing the amino acid sequence of G_o (SEQ ID NO: 2) or an APP-associating region of G_o (SEQ ID NOs: 3, 4, or 5), in the presence of a candidate compound; and
either (i) determining whether the candidate
10 compound interferes with (i.e., inhibits partially or completely) the association of the first and second molecules, or (ii) determining whether the candidate compound interferes with the activation of the second molecule by the first molecule, such interference being
15 an indication that the candidate compound is a potential therapeutic useful for treating or preventing Alzheimer's disease. The determining step may be accomplished by, for example, immunoprecipitating the first molecule with an antibody specific for APP, and detecting the presence
20 or amount of the second molecule which co-precipitates with the first molecule. Alternatively, the second molecule can be immunoprecipitated with an antibody specific for G_o , following which the presence or amount of the first molecule which co-precipitates with the
25 second molecule is determined. Where activation is the criterion being measured, the determination step may be accomplished by contacting the second molecule with a substrate which is or includes GTP or an analog of GTP [such as GTPyS or Gpp(NH)p], and detecting or measuring
30 the binding of the substrate to the second molecule, wherein such binding is evidence of activation of the second molecule by the first molecule. In preferred embodiments, the contacting step is carried out in a cell-free system; the Mg^{2+} concentration at which the
35 contacting step is carried out is between approximately

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1x10⁻⁷ and 1x10⁻² M, and the first molecule includes the cytoplasmic tail portion of APP₆₉₅ from residues 649 to 695 (SEQ ID NO: 6) and/or the membrane-spanning portion of APP₆₉₅ from residues 639 to 648 (SEQ ID NO: 7) (the entire membrane-spanning segment of APP₆₉₅ being from residues 625 to 648, SEQ ID NO: 8); the first molecule more preferably includes substantially all of APP (SEQ ID NO: 9). (Alternatively, the corresponding functional regions of APP₇₅₁ or APP₇₇₀, or any other APP, may be used.) The second molecule preferably contains two or three of the putative APP-associating regions referred to above, and may also contain one or more of the GTP-binding regions of G_o, corresponding to residues 35 to 50 (SEQ ID NO: 10), residues 201 to 218 (SEQ ID NO: 29), or residues 263 to 274 (SEQ ID NO: 30) of G_{o1} [Kaziro, "Structure of the genes coding for the α subunits of G proteins", Ch. 1 in ADP-ribosylating Toxins and G proteins (Moss, J., and Vaughan, M. eds.) pp189-206, American society for Microbiology, Washington, D.C. (1988)], and more preferably contains substantially all of G_o (SEQ ID NO: 2).

The invention also includes a system (e.g., a cell-free *in vitro* system) for screening candidate Alzheimer's disease therapeutics, which system includes a first polypeptide containing a sequence essentially identical to that of peptide 20 (SEQ ID NO: 1), and a second polypeptide containing a sequence essentially identical to one, two or three of the putative APP-associating regions of G_o (SEQ ID NOs: 3, 4, and 5); the system may also include a means for detecting either (a) the association of the first polypeptide with the second polypeptide, or (b) the activation of the second polypeptide by the first polypeptide. The first polypeptide may conveniently be anchored to a solid material (e.g., a cellular membrane, a polystyrene

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surface, or a standard matrix material), or may be in a phospholipid vesicle. It may include a sequence essentially identical to the membrane-spanning region of APP, and/or a sequence essentially identical to the entire cytoplasmic tail of APP. The second molecule preferably contains the GTP-binding domain of G_o , and more preferably contains the entire sequence of G_o .

The invention also features a method for diminishing the activation of G_o in a neuronal cell by treating the cell with a compound, such as a peptide fragment of G_o or of the cytoplasmic tail of APP, which blocks association of neuronal G_o with, and/or activation of neuronal G_o by, the cytoplasmic tail of APP. The cell may be so treated *in vivo* (i.e., in an animal, e.g. a mammal such as a human or other primate, cow, horse, pig, sheep, goat, dog, cat, rat, mouse, guinea pig, hamster, or rabbit) or *in vitro*. This method may be used to prevent or treat the symptoms of Alzheimer's disease in a patient. Such a compound may include, for example, a peptide having fewer than 50 amino acids (preferably 40 or fewer, and more preferably 30 or fewer), and containing the sequence of peptide 20. Also within the invention is a DNA molecule (e.g., a plasmid or viral DNA) encoding such a peptide, and a therapeutic composition containing, in a pharmaceutically acceptable carrier, either the peptide or the DNA molecule.

In another aspect, the invention features a method for identifying a ligand for which APP is a receptor, which method includes the steps of

- 30 providing an APP molecule, the cytoplasmic tail of which is accessible to a molecule of G_o ;
- contacting a candidate compound with the extracellular domain of the APP molecule; and
- detecting either (a) association of G_o with the APP molecule, (b) dissociation of G_o from the APP

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molecule, or (c) activation of G_o by the APP molecule, such association, dissociation, or activation being evidence that the candidate compound is a ligand of APP.

Other features and advantages of the invention will be apparent from the detailed description set forth below, and from the claims.

Brief Description of the Drawings

Fig. 1(a) is a schematic diagram illustrating the structural organization of APP. The hatched box contains the sequence of the β/A_4 protein; the black box contains the so-called "Peptide 20" or couplone sequence; filled circles are N-glycosylation sites. The numbers designate amino acid sequence numbers corresponding to APP₆₉₅.

Fig. 1(b) is a bar graph illustrating the effects of synthetic APP peptides on G_o . In (b), (d), (e) and (f), values represent the mean \pm S.E. of three experiments.

Fig. 1(c) is a graph illustrating the time course of the action of peptide 20 on G_o . Values represent the mean of three experiments. Since the S.E. was $< 5\%$ of each value in this figure, the error bars are not indicated.

Fig. 1(d) is a graph illustrating the effects of peptide 20 variants on G_o .

Fig. 1(e) is a graph illustrating the effect linkage with a transmembrane region has on the action of peptide 20 on G_o .

Fig. 1(f) is a graph illustrating the effect of pertussis toxin on peptide 20-induced stimulation of GTP- γ S binding to G_o .

Figs. 2a-2d is a set of SDS-PAGE gels analyzed by immunoblotting, which illustrate the immunoprecipitation of APP and G_o by an anti-APP antibody from brain membranes. (a) Immunoprecipitation of APP by 22C11.

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- (b) Immunoprecipitation of G_o by 22C11. (c) Effect of Mg^{2+} on the immunoprecipitation of G_o by 22C11.
(d) Effect of peptide 20 on 22C11-induced precipitation of $G_{o\alpha}$ (left) and APP (right). Each of the results
5 presented in this figure was reproduced at least three times.

Fig. 3a is a schematic diagram of the construction method used to prepare recombinant mutant APP cDNAs. Regions labeled ATG, TAA, TGA signify original
10 translation and termination sites and a newly inserted termination site, respectively.

Fig. 3b is a schematic diagram comparing the structures of authentic APP₆₉₅ and the two recombinant mutant APP polypeptides, ΔN and ΔC .

- 15 Fig. 3c is an immunoblot analysis of Sf9 membranes using anti-Alz 90, 1C1, and 4G5.

Fig. 3d is an immunoblot analysis of the 22C11-precipitate from an Sf9 membrane- G_o reconstitution mixture.

- 20 Fig. 3e is an immunoblot illustrating dissociation of G_o from APP by activation of G_o . Each of the results presented in Figs. 3c-e was reproduced at least three times.

- Fig. 4a is the cDNA sequence and deduced amino
25 acid sequence of $G_{o1\alpha}$ (Strathmann et al., Proc. Natl. Acad. Sci. USA 87:6477-6481, 1990) (SEQ ID NO: 2).

Fig. 4b is the cDNA sequence and deduced amino acid sequence of $G_{o2\alpha}$ (Strathmann et al.) (SEQ ID NO: 28).

Detailed Description

- 30 It was previously shown that the insulin-like growth factor II receptor (IGF-IIR) couples directly to the G protein referred to as G_i (Nishimoto et al., J. Biol. Chem. 264:14029-14038, 1989) via a 14-residue section of the cytoplasmic tail of IGF-IIR, Arg²⁴¹⁰-Lys²⁴²³

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(Okamoto et al., Cell 62:709-717, 1990; Okamoto et al., Proc. Natl. Acad. Sci. U.S.A. 88:8020-8023, 1991). The structural determinants for the G_i-activating function in IGF-IIR were defined as (i) two basic residues at the N-terminal region of the amino acid sequence, and (ii) a C-terminal motif of B-B-X-B or B-B-X-X-B (where B is a basic residue and X is a non-basic residue) (Okamoto et al., Cell 62:709-717, 1990). To assess whether APP might function as a G protein-coupled receptor, the amino acid sequence of human APP₆₉₅ was examined for regions of less than 26 residues which satisfy (i) and (ii). The sequence His⁶⁵⁷-Lys⁶⁷⁶ is the only such region in the cytoplasmic domain of APP₆₉₅. In two other isoforms of APP, APP₇₅₁ (Ponte et al., Nature 331:525-527, 1988; Tanzi et al., Nature 331:528-530, 1988) and APP₇₇₀ (Kitaguchi et al., Nature 331:530-532, 1988), as well as in mouse APP₆₉₅ (Yamada et al., Biochem. Biophys. Res. Commun. 149:665-671, 1987), this sequence is completely conserved.

Preparation of peptides

A peptide corresponding to the His⁶⁵⁷-Lys⁶⁷⁶ region of APP [HHGVVEVDAAVTPEERHLSK (SEQ ID NO: 1)] was synthesized and purified by standard methods using solid phase synthesis; this peptide is referred to as "peptide 20". Similarly prepared were peptides corresponding to other regions of APP₆₉₅: APP(1-10), MLPGLALLLL (SEQ ID NO: 11); APP(597-606), DAEFRHDSGY (SEQ ID NO: 12); APP(677-695), MQQNGYENPTYKFFEQMQN (SEQ ID NO: 13); and APP(639-648), TVIVITLVML (SEQ ID NO: 7), a portion of the transmembrane region of APP; as well as the following variants of peptide 20: HGVVEVDAAVTPEERHLSK (H-deleted, SEQ ID NO: 14); GVVEVDAAVTPEERHLSK (HH-deleted, SEQ ID NO: 15); HHGVVEVDAAVTPEE (RHLSK-deleted, SEQ ID NO: 16);

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KQYTSIHGGVVEVDAAVTPEERHLSK (KQYTSI-added, SEQ ID NO: 17);
and TVIVITLVMLHGGVVEVDAAVTPEERHLSK (transmembrane region-
connected peptide 20; SEQ ID NO: 18).

Peptides were purified by HPLC to greater than 95%
5 purity, and were used immediately after synthesis.

Materials and Methods.

Trimeric G_o was purified to homogeneity from bovine brain as described (Katada et al., FEBS Lett. 213:353-358, 1987). This G_o preparation was stored in 20
10 mM Hepes/NaOH (pH 7.4), 1 mM EDTA, and 0.7% CHAPS, and diluted ≥ 10 fold for assays. $G_{13\alpha}$, which was used in combination with 1.5-fold concentrated $G\beta\gamma$ (Okamoto et al., Natl. Acad. Sci. U.S.A. 88:8020-8023, 1991), was prepared as described by Morishita et al., Biochim.
15 Biophys. Acta 161:1280-1285, 1989. Low molecular weight G proteins were prepared as described by Matsui et al., J. Biol. Chem. 263:11071-4, 1988; $G\beta\gamma$ was purified from bovine brain as set forth in Katada et al., FEBS Lett. 213:353-358, 1987.

20 GTP γ S binding to G_o was assayed in a buffer containing 50 mM Hepes/NaOH (pH 7.4), 100 μ M EDTA, 120 μ M $MgCl_2$, and 60 nM [^{35}S]GTP γ S (DuPont-New England Nuclear) at 37°C, and the fraction of total G_o bound to GTP γ S was measured as described (Okamoto et al., Cell 62:709-717,
25 1990). GTP γ S binding to peptides was negligible. The total amount of G_o in a given preparation was defined as the saturation amount of GTP γ S bound to G_o following a 30-min incubation of G_o with 10 mM Mg^{2+} and ≥ 60 nM GTP γ S at 30°C.

30 Reconstitution of G_o into phospholipid vesicles was accomplished with 1 mg/ml of phosphatidylcholine, using the gel filtration method (Nishimoto et al., J. Biol. Chem. 264:14029-14038, 1989). In a final

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incubation for GTP γ S binding, 5 nM of reconstituted G $_o$ was used.

For experiments exploring the effect of Mg $^{2+}$, the Mg $^{2+}$ concentration was set by using Mg-EDTA buffer
5 (Birnbaumer et al., J. Eur. J. Biochem. 136:107-112, 1983).

Bovine brain membranes, prepared as described (Katada et al., FEBS Lett. 213:353-358, 1987) and suspended in buffer A [10 mM Hepes/NaOH (pH 7.4), 1 mM
10 EDTA, 10 mM acetic acid, and 250 mM sucrose, plus a mixture (termed "PAL") of 2 mM PMSF, 20 μ g/ml aprotinin, and 20 μ M leupeptin], were centrifuged and the pellet was solubilized for 1 h at 4°C in buffer B (10 mM Hepes/NaOH (pH 7.4), 1 mM EDTA, 120 mM NaCl, 0.5% CHAPS, and PAL).
15 Following centrifugation of the material at 15000 rpm for 1 h, the supernatant (500 μ g protein, unless specified) was incubated in buffer C (20 mM Hepes/NaOH (pH 7.4), 1 mM EDTA, 120 mM NaCl, and PAL) and 2% BSA with 22C11-coated protein G-Sepharose, which had been prepared by
20 incubating protein G-Sepharose (Pharmacia) with anti-APP monoclonal antibody 22C11 (Boehringer Mannheim) for 1 h at 4°C. An antibody concentration of ≥ 2 μ g/ml was found to saturate precipitation of APP and G $_o$, so 2 μ g/ml was the concentration used for immunoprecipitation studies.
25 As a control, 2 μ g/ml of rabbit IgG was used. After overnight shaking at 4°C, the immunoprecipitated sample was centrifuged at 5000 rpm for 5 min. The pellet was washed three times with ice-cold buffer C and the final pellet was applied to SDS-PAGE. Electrophoretic transfer onto a
30 PVDF sheet was performed as described (Okamoto et al., J. Biol. Chem. 266:1085-1091, 1991). After blocking with PBS containing 2% skim milk and 1% BSA, the sheet was incubated with the first antibody [1 μ g/ml of 22C11; 1/1000 dilution of anti-G $_{\alpha}$ monoclonal antibody GC/2
35 (DuPont-New England Nuclear); 1/1000 dilution of 1C1, a

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monoclonal antibody against the C-terminal peptide 677-695 of APP₆₉₅] for 4 h, and then exposed to horseradish peroxidase-conjugated goat IgG reactive for mouse or rabbit immunoglobulins for 2-4 h at room temperature.

- 5 The antigenic bands were detected with an ECL detection kit (Amersham). YL1/2 (SERA Lab), an anti-tubulin antibody, was used at 1:500 dilution for immunodetection.

Effects of synthetic APP peptides on G proteins.

- In the experiment shown in Fig. 1(b), 10 nM G_o was
- 10 incubated with water or 100 μM of each peptide for 2 min, and the amount of GTPγS bound to G_o at the end of this period was measured. In the experiment shown in Fig. 1(c), 10nM G_o was incubated with water (○) or 100 μM peptide 20 (SEQ ID NO: 1) (●), and GTPγS binding was
 - 15 measured at the indicated times. From Fig. 1(d), it can be seen that peptide 20 (SEQ ID NO: 1) stimulated the rate constant of GTPγS binding to G_o in a dose-dependent manner, whereas Fig. 1(b) shows that peptides from other regions of APP₆₉₅ were ineffective. GTPγS binding to G_o
 - 20 in the presence or absence of peptide 20 (SEQ ID NO: 1) obeyed first-order kinetics according to the equation

$$\ln [(Bt-B)/Bt] = -k_{app}t$$

- (B is the binding at time t; Bt is the total binding observable at infinite time; and k_{app} is the rate constant
- 25 for GTPγS binding). The ability of peptide 20 (SEQ ID NO: 1) to activate G_o was gradually decreased during storage at either -4°C or -20°C.

- Studies using structural variant peptides suggest that both the N-terminal basic residues and the C-
- 30 terminal B-B-X-X-B motif play essential roles in the G_o-activating function of peptide 20 (SEQ ID NO: 1) [Fig. 1(d)]. In this experiment, 10 nM G_o was incubated with various concentrations of HHGVVEVDAAVTPEERHLSK (peptide 20, SEQ ID NO: 1; □), HGVVEVDAAVTPEERHLSK (H-deleted, SEQ

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ID NO: 14; ◇), GVVEVDAAVTPEERHLSK (HH-deleted, SEQ ID NO: 15; □), HHGVVEVDAAVTPEE (RHLSK-deleted, SEQ ID NO: 16; ◆), or KQYTSIHGGVVEVDAAVTPEERHLSK (KQYTSI-added, SEQ ID NO: 17; ■), and GTPγS binding to G_o at 2 min. was measured. Fig. 1(d) indicates which aspects of primary structure determine the G_o -activator function of peptide 20 (SEQ ID NO: 1). Deletion of either one or both of the N-terminal His residues nullified G_o -activator function of the peptide. The peptide (SEQ ID NO: 16) in which the C-terminal five residues of peptide 20 (SEQ ID NO: 1) has been deleted is several times less potent than peptide 20 (SEQ ID NO: 1).

As illustrated in Fig. 1(e), G_o reconstituted in phospholipid vesicles was incubated with transmembrane region-connected peptide 20 (TVIVITLVMLHHGVVEVDAAVTPEERHLSK, SEQ ID NO: 18; □) or the partial sequence of the APP transmembrane domain alone (TVIVITLVML, SEQ ID NO: 7; □). Transmembrane region-connected peptide 20 (SEQ ID NO: 18) was also incubated with G_o in the absence of phospholipids and the presence of 0.07% CHAPS (◆). The transmembrane region-connected peptide 20 (SEQ ID NO: 18) stimulated G_o reconstituted in phospholipid vesicles with a potency 10 times greater than that of peptide 20 (SEQ ID NO: 1). The transmembrane region alone (SEQ ID NO: 7) was without effect on G_o . In the absence of phospholipids, transmembrane region-connected peptide 20 (SEQ ID NO: 18) showed an effect on G_o no more potent than peptide 20 (SEQ ID NO: 1). Therefore, the stimulatory action of this transmembrane region-connected peptide (SEQ ID NO: 18) is attributed to the peptide 20 (SEQ ID NO: 1) sequence; the potentiating effect of the transmembrane region may be exerted by interactions with phospholipids.

In the experiment shown in Fig. 1(f), ADP-ribosylation of G_o was accomplished by incubating G_o

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reconstituted in phospholipid vesicles with 10 $\mu\text{g/ml}$ preactivated pertussis toxin in the presence of 10 μM NAD for 15 min at 30°C as described (Okamoto et al., Cell 62:709-717, 1990). Preactivation of pertussis toxin (Funakoshi, Japan) was carried out by treating the toxin with 100 μM ATP and 1 mM DTT for 10 min at 30°C. Reconstitution of G_o into phospholipid vesicles was accomplished with 1 mg/ml phosphatidylcholine (Sigman, P-5638) at a final G_o concentration of 50.2 nM in a buffer containing 20 mM Hepes/NaOH (pH 7.4), 0.1 mM EDTA, 1 mM DTT, and 100 mM NaCl by the gel filtration method (Nishimoto et al., J. Biol. Chem. 264:14029-14038, 1989). In a final incubation for GTP γ S binding, 5 nM of reconstituted G_o was used. Increasing concentrations of peptide 20 (SEQ ID NO: 1) were incubated for 2 min with G_o reconstituted in phospholipid vesicles which had been treated with pertussis toxin in the presence (\blacklozenge) or absence (\square) of NAD, and GTP γ S binding to G_o was measured.

Although peptide 20 (SEQ ID NO: 1) produced 2-3 fold stimulation of GTP γ S binding to G_o in the mid-range of Mg^{2+} concentrations, the effect of peptide 20 (SEQ ID NO: 1) could not be observed at low (≤ 100 nM) or high (≥ 10 mM) Mg^{2+} concentrations.

Peptide 20 (SEQ ID NO: 1) had little effect on G proteins other than G_o : G_{i1} , G_{i2} , G_{i3} , G_s , c-Ki-ras p21 and smg p25A were not stimulated by this peptide (data not shown). Thus, peptide 20 (SEQ ID NO: 1) activates G_o in a receptor-like manner, suggesting that APP interacts directly with G_o through the peptide 20 (SEQ ID NO: 1) region.

Coprecipitation of APP and G_o

In an effort to determine whether APP is linked to G_o in a native membrane environment, the coprecipitation studies shown in Fig. 2a were performed. Solubilized membranes of bovine brain were first immunoprecipitated

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by monoclonal anti-APP antibody 22C11, and the immunoprecipitate was then probed by immunodetection with 22C11 (Lane 2) or 1C1, a monoclonal antibody against the C-terminal peptide₆₇₇₋₆₉₅ of APP (SEQ ID NO: 13; Lane 4).
5 Lanes 1 and 3 of Fig. 2a indicate the controls in which either no solubilized membranes were included (Lane 1), or rabbit IgG was used for the precipitation step instead of antibody 22C11 (Lane 3). In each control, immunodetection was performed with 22C11. The 55-kDa and
10 25-kDa bands seen in Lanes 1 and 2 may be heavy and light chains of the 22C11 used for precipitation, which reacted with an anti-mouse IgG antibody during immunodetection. The precipitate by control rabbit IgG contained no detectable APP. Although the 100 kD molecular size of
15 APP appears here to be slightly less than the 110-130 kD reported (Weidemann et al., Cell 57:115-126, 1989), the precipitated form is unlikely to be an extracellular fragment of APP, because 1C1 recognizes this 100-kDa band.

20 In the experiment illustrated in Fig. 2b, coprecipitation of various G proteins with APP was investigated. Bovine brain membrane preparations were immunoprecipitated with 22C11; the immunoprecipitated proteins were subjected to SDS-PAGE and immunoblotted
25 with the indicated anti-G protein antisera (1/1000 dilution). Lane 2: GC/2, anti-G_oα antiserum; lane 3: GC/2 plus 1 μg/ml of purified G_o; lane 4: GA/1, common Gα antiserum; lane 5: AS/7, anti-Giα antiserum; lane 6: MS/1, common Gβ antiserum. Lane 1 shows a control
30 immunoblot with GC/2, in which a buffer solution rather than the bovine brain membrane preparation was immunoprecipitated with 22C11. Lane 7 indicates immunoblotting with GC/2 of the precipitate resulting from immunoprecipitation of brain membranes with control
35 rabbit IgG, rather than 22C11. The identity of the 39-

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kDa protein in lane 2 as G_o was verified by its absence in the non-membrane control (lane 1); by its staining with another $G_o\alpha$ -specific antibody, $\alpha GO1$ (Morishita et al., Eur. J. Biochem. 174:7-94, 1988) (data not shown);

5 and by a diminution of staining of this band in the presence of excess soluble G_o (lane 3). The 22C11-precipitate also contained immunoreactivity of $G\beta$ in a doublet at 35-36-kDa (lane 6). The 22C11-precipitate did not react with an anti- $G_{i\alpha}$ antibody AS/7 (lane 5). The

10 antibody GA/1 detected only a 39-kDa band in the 22C11-precipitate (lane 4). The control rabbit IgG immunoprecipitate did not produce anti- G_o -immunoreactive bands corresponding to either APP or G_o (lane 7). These experiments indicate that the 22C11-precipitate from

15 brain membranes contains APP immunoreactivity at 100 kDa, $G_o\alpha$ immunoreactivity at 39 kDa, and $G\beta$ immunoreactivity in a doublet at 35-36 kDa, but no detectable immunoreactivity indicating the presence of $G_{i\alpha}$ or other heterotrimeric G proteins. A tubulin antibody, YL1/2,

20 did not stain the 22C11-precipitate (data not shown).

In the experiment shown in Fig. 2c, the effect of Mg^{2+} concentration on co-precipitation of G_o with anti-APP antibody was studied. 100 μg of solubilized brain membranes were precipitated by 22C11 in the presence of

25 various Mg^{2+} concentrations controlled with Mg-EDTA buffer (Birnbaumer et al., J. Eur. J. Biochem. 136:107-112, 1983). The precipitates were analyzed by immunoblotting with GC/2. The control lane indicates the results of precipitation of brain membranes by rabbit IgG followed

30 by immunodetection with GC/2. In the absence of Mg^{2+} , G_o was less efficiently co-precipitated by 22C11. Mg^{2+} concentrations between 1 μM and 1 mM resulted in maximal immunoprecipitation of G_o . At concentrations > 10 mM, relatively little G_o was precipitated. In contrast,

35 immun precipitation of APP by 22C11 was not affected by

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Mg²⁺ concentration (data not shown). These results indicate that, while Mg²⁺ is not absolutely required for complex formation by APP and G_o, the concentration of Mg²⁺ does strongly influence complex formation. A mid range of Mg²⁺ concentration was found to facilitate APP-G_o association.

Fig. 2d illustrates the results of an experiment indicating that peptide 20 (SEQ ID NO: 1) prevents the 22C11-mediated co-precipitation of G_o, whereas it did not affect the precipitation of APP by 22C11. In contrast, a control peptide (SEQ ID NO: 13) representing a segment of APP different from that represented by peptide 20 (SEQ ID NO: 1) had no discernable effect on 22C11-mediated co-precipitation of G_o. In this experiment, solubilized brain membranes were incubated with 22C11-coated beads in the presence of 10 μM peptide 20 (SEQ ID NO: 1; 2nd and 5th lanes) or 10 μM of the control peptide, peptide₆₇₇₋₆₉₅ of APP (SEQ ID NO: 13; 3rd and 6th lanes), or in the absence of both of these peptides (1st and 4th lanes). In this experiment, an anti-mouse IgG antibody different from that used in (a) was employed.

Precipitation of G_o reconstituted with recombinant APP-antibody complex

A baculovirus DNA encoding full-length APP₆₉₅ (SEQ ID NO: 9) was prepared as outlined in Fig. 3a. Authentic mouse APP₆₉₅ cDNA (SEQ ID NO: 9) was provided by Dr. Yoshiyuki Sakaki (University of Tokyo, Japan) (Yamada et al., Biochem. Biophys. Res. Commun. 149:665-671, 1987) in the vector pUC18. The HindIII-BamHI fragment containing the entire coding region was initially subcloned into the vector pBR322 (pBR-APP). A single BamHI site was inserted immediately before the ATG codon of the HindIII-SphI fragment. This BamHI site was inserted to permit efficient expression of the encoded APP protein in

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baculovirus-infected cells. The BamHI site-inserted APP₆₉₅-coding DNA (BamHI-APP₆₉₅) was constructed from the HindIII-SphI fragment and pBR-APP, utilizing their internal KpnI sites, and subcloned into pUC18. By using

5 BamHI-APP₆₉₅ as template, two truncation mutants were generated and subcloned into pUC18. These mutants possess an insertion of two TGA codons immediately before (ΔN) or after (ΔC) the peptide 20 sequence. Each BamHI-BamHI fragment of these respective APP-variation-encoding

10 pUC18 plasmids was inserted into the baculovirus transfer/expression vector pVL1393 (Invitrogen). The entire region that had been through a single-stranded intermediate was sequenced to confirm the absence of unwanted nucleotide changes. New insertions were

15 generated by oligonucleotide-directed mutagenesis with a kit (Takara) by the method of Kunkel et al. (Meth. Enzymol. 154:367-382, 1987). For the insertion of a BamHI site, a restriction fragment encoding the ATG start codon was subcloned into the vector M13mp18 and a single

20 stranded template was generated. An oligonucleotide primer (CCACGCAGGATCACGGGATCCATGCTGCCCAGCTTG; SEQ ID NO: 19) was used to introduce GGATCC (SEQ ID NO: 20) immediately before the start codon. Following primer extension, the phage was used to transform E. coli strain

25 JM109. Plaques were selected and single stranded DNA was sequenced. A restriction fragment containing the mutated region was subcloned into pBR-APP. For the insertion of the stop codons, oligonucleotide primers [CAGTACACATCCATCTGATGACATCATGGCGTGGTG (SEQ ID NO: 21) and

30 CGCCATCTCTCCAGTGATGAATGCAGCAGAACGGA (SEQ ID NO: 22)] and the M13mp19 vector were used to introduce two sequential TGA stop codons. Using the method of Summers and Smith (Summers et al., Tex. Agric. Exp. Stn. Bull. 1555, 1987), baculoviruses incorporating these APP cDNAs were

35 generated using selection by immunoblot analysis with

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22C11, and recovered by infecting Sf9 cells (Invitrogen). Four days after treatment of Sf9 cells with the viruses, cells were homogenized and suspended in buffer A. After the solubilization of the pellet with buffer B, the

5 supernatant (100 μ g) was mixed overnight with 22C11-coated protein G-Sepharose in buffer C plus 2% BSA at 4°C on a shaker. After centrifugation, the precipitated beads were incubated with purified G₀ (1 μ g) in buffer C supplemented with 1.1 mM MgCl₂ and 2% BSA for 8-24 h at

10 4°C on a shaker. After washing four times with ice-cold buffer C, the centrifugation precipitate was subjected to SDS-PAGE, electroblotting, and immunodetection with the first antibodies (1 μ g/ml of 22C11; 10 μ g/ml of anti-Alz 90; 1/1000 dilution of 1C1; 1/500 dilution of 4G5; 0.1

15 μ g/ml of α GO1) and the second goat anti-mouse or anti-rabbit IgGs conjugated with HRP. (Immunodetection of 1C1 and 4G5, both of which are mouse IgM (κ), was accomplished using as second antibody a mixture of HRP-conjugated anti-rabbit IgG, rabbit anti-mouse IgM and

20 rabbit anti-mouse κ antibodies.) The three APP constructs prepared as described above are compared in the schematic diagram of Fig. 3b. The polypeptides encoded by all three constructs retain the entire transmembrane and extracellular domains of APP;

25 while Δ N (SEQ ID NO: 23) lacks all of the peptide 20 residues as well as the sequence on the carboxy terminal side of the peptide 20 region, Δ C (SEQ ID NO: 24) retains the peptide 20 sequence and is missing only the latter sequence.

30 Sf9 cells were infected, using standard methods, by recombinant baculoviruses encoding full length APP₆₉₅ cDNA (SEQ ID NO: 9), APP₁₋₆₅₆ cDNA (Δ N; SEQ ID NO: 23), or APP₁₋₆₇₆ cDNA (Δ C; SEQ ID NO: 24). In uninfected Sf9 cells, no immunoreactivity for anti-APP or anti-G₀

35 antibodies was detected (data not shown). The membranes

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of Sf9 cells infected with the baculoviruses encoding APP₆₉₅ (SEQ ID NO: 9), ΔN (SEQ ID NO: 23), and ΔC (SEQ ID NO: 24) genes (referred to as Sf9-APP₆₉₅, Sf9-ΔN, and Sf9-ΔC, respectively) were found to express, respectively, 130-, 120- and 130-kDa proteins reactive with antibody 22C11 (Fig. 3d, right side). The Sf9-APP₆₉₅ cells expressed APP at $\approx 0.1\%$ of the total membrane protein. When the membranes of the three types of infected cells were immunoprecipitated with antibody Anti-Alz 90 (Boehringer Mannheim), a mouse monoclonal antibody specific for an epitope corresponding to residues 551-608 of APP (SEQ ID NO: 25; a section of APP that is within the extracellular domain), 130-kDa, 120-kDa, and 130-kDa proteins were recognized in Sf9-APP₆₉₅, Sf9-ΔN, and Sf9-ΔC cells, respectively (Fig. 3c, top panel). Membranes from all three types of infected cells showed approximately equivalent reactivity to the antibody, indicating that at least this portion of the extracellular domain was intact on each of the three and that all three cell types express approximately equal amounts of recombinant protein. When the antibody used was 1C1, a mouse monoclonal prepared against a peptide corresponding to residues 677-695 of APP (SEQ ID NO: 13), only Sf9-APP₆₉₅ membranes were reactive, indicating that the region corresponding to the C-terminal portion of the cytoplasmic domain is missing from both ΔN (SEQ ID NO: 23) and ΔC (SEQ ID NO: 24) (Fig. 3c, middle panel). When the antibody used was 4G5, a mouse monoclonal antibody raised against a peptide corresponding to residues 657-676 of APP (SEQ ID NO: 1; the peptide 20 region of the cytoplasmic domain), 130 kDa bands from both Sf9-APP₆₉₅ and Sf9-ΔC membranes reacted with the antibody, but Sf9-ΔN membranes did not, a demonstration that ΔN (SEQ ID NO: 23) but not ΔC (SEQ ID NO: 24) lacks the peptide 20 region of APP (Fig. 3c, bottom panel).

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These experiments clearly indicate that the expressed proteins are recombinant APP₁₋₆₉₅ (SEQ ID NO: 9), APP₁₋₆₅₆ (SEQ ID NO: 23), and APP₁₋₆₇₆ (SEQ ID NO: 24), respectively, as designed.

- 5 The 22C11-precipitates from these Sf9 membranes expressing various forms of APP were exposed to purified G_o, reprecipitated with 22C11, and subjected to immunoblot analysis using anti-G_oα antibody αGO1 (Fig. 3d, left four lanes) and by 22C11 (right four
10 lanes). αGO1 (Morishita et al., Eur. J. Biochem. 174:87-94, 1988) was provided by Dr. Tomiko Asano; similar results were obtained when antibody GC/2 was substituted. The control lanes are 22C11-precipitate exposed to G_o in the absence of Sf9 membranes.
- 15 Approximately 1/10-1/20 (0.05-0.1 μg/tube) of the reconstituted G_o was precipitated, together with a comparable amount (≈0.1 μg/tube) of APP. Easily detectable amounts of G_oα were present in the final precipitate when G_o was mixed with 22C11-precipitates
20 from Sf9-ΔC or Sf9-APP₆₉₅ membranes, but essentially no G_oα was found in the final precipitate from Sf9-ΔN membranes. Thus, formation of an APP-G_o complex requires the peptide 20 region, residues 657-676 (SEQ ID NO: 1).

- In the experiment illustrated in Fig. 3e, 22C11-
25 precipitates from Sf9-APP₆₉₅ membranes (100 μg protein each) were incubated with activated G_o (lanes 2 and 4) or unactivated G_o (lanes 1 and 3); the final precipitates (left panel) and supernatants (right panel) were analyzed by simultaneous immunoblotting with 22C11 and αGO1
30 antibodies. Activation of G_o was carried out by incubating G_o in 20 mM Hepes/NaOH (pH 7.4), 1 mM EDTA, 2 mM MgCl₂, and 1 μM GTPγS overnight at room temperature. When G_o was incubated with GTPγS, no G_oα associated with the APP-22C11 complex (Fig. 3e), suggesting that the

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activation state of the G protein regulates APP-G_o association.

This study suggests that APP functions as a receptor coupled to G_o through the G_o-activator cytoplasmic domain His⁶⁵⁷-Lys⁶⁷⁶ (SEQ ID NO: 1). APP has a point mutation in at least one form of familial Alzheimer's disease (Goate et al., Nature 349:704-706, 1991). A structural alteration of APP is therefore thought to be one cause of Alzheimer's disease, although it remains unknown how the mutation might produce the disease. One novel possibility suggested by this study is that the cytoplasmic, C-terminal fragment of APP is pathogenic. It has been suggested (Abraham et al., Biotechnology 7:147-153, 1989; Shivers et al., EMBO J. 7:1365-1370, 1988; Kametani et al., Biomedical Research 10:179-183, 1989) that the residual C-terminal portion of APP may remain in the cell membrane after abnormal cleavage of APP to produce β /A4 protein in Alzheimer's disease neurons. By analogy with the oncogenic transformation of c-erb B into v-erb B, such a structural alteration of APP may alter its function and prompt APP to constitutively activate G_o. This hypothesis is consistent with the study (Yanker et al., Science 245:417-420, 1989) indicating that recombinant expression of the C-terminal 105-residue portion of APP in neuronal cells evokes cell death, and with the reports that G_o activity is linked to neuronal growth cone motility (Strittmatter et al., BioEssays 13:127-134, 1990), axon and dendrite formation (Granneman et al., J. Neurochemistry 54:1995-2001, 1990), and memory (Guillen et al., EMBO J. 9:1449-1455, 1990). This study suggests that Alzheimer's disease is a disorder of an APP-G_o signalling system caused by structural alterations of APP.

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Example 1

The screening method of the invention can be carried out as follows:

The assay used can be a very simple cell-free
5 assay employing a first polypeptide consisting essentially of the couplone, or G_o -binding portion, of APP (SEQ ID NO: 1) and a second polypeptide consisting essentially of an APP-binding portion of G_o . This APP-binding portion of G_o may be the 15-residue segment
10 identified as the anticouplone portion of G_o (SEQ ID NO: 3), or it may be one or both of the two flanking regions, residues 1-3 (SEQ ID NO: 4) and residues 19-36 (SEQ ID NO: 5) of G_o . Alternatively, longer portions, or all, of APP and/or G_o can be used, or the appropriate
15 portions of APP and/or G_o can be linked to other polypeptides to form hybrid polypeptides with characteristics (such as altered immunoreactivity or enzymatic activity) that would improve detection of the endpoint of the assay. The assay is carried out by
20 contacting the APP-based polypeptide with the G_o -based polypeptide in the presence of a candidate compound, in parallel with a control assay containing no candidate compound, and determining whether the candidate compound inhibits co-immunoprecipitation of the first and second
25 polypeptides (using either an antibody specific for the first polypeptide or an antibody specific for the second polypeptide). Alternatively, activation of the second (G_o) polypeptide may be the measured criterion: if so, the second polypeptide must include the GTP-binding
30 region of G_o (SEQ ID NO: 10), and GTP or an appropriate non-hydrolyzable analog thereof (such as GTP γ S or Gpp(NH)p) must be included in the assay. The assay may also be carried out using phospholipid vesicles prepared by standard methods (e.g., as described by Nishimoto et
35 al., J. Biol. Chem. 264:14029-14038, 1989), provided that

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the first (APP) polypeptide includes a region of hydrophobic amino acids [such as all (SEQ ID NO: 8) or a portion (e.g., SEQ ID NO: 7) of the transmembrane region of APP) that permit it to be anchored in the phospholipid bilayer. Alternatively, the assay may be carried out using intact cells or red cell ghosts which contain APP and G_o , or appropriate portions thereof. The cells may express the first and second polypeptides naturally or by virtue of genetic engineering, or the polypeptides may be introduced directly into the cells or ghosts by standard means.

Example 2

The progress of Alzheimer's disease may be halted or reversed by treating a patient with a compound which diminishes the activation of neural G_o by truncated APP. Such a compound may be identified in a screening assay as described above, or may consist essentially of a polypeptide containing the amino acid sequence of (a) the couplone region of APP (SEQ ID NO: 1), (b) the anticouplone region of G_o (SEQ ID NO: 3), or (c) the APP-associating region(s) of G_o (SEQ ID NO: 4 and/or 5), or a combination of (b) and (c). Such polypeptides may be produced in quantity by standard recombinant means, or by standard synthetic techniques. To minimize proteolytic degradation *in vivo*, the carboxy and amino termini may be derivatized (e.g., with ester or amide groups), some or all of the amino acids may be replaced with D-amino acids, or particularly sensitive peptide linkages may be substituted with non-peptide bonds using standard methodology. To improve penetration of the blood-brain barrier (BBB), the polypeptides may be altered to increase lipophilicity (e.g., by esterification to a bulky lipophilic moiety such as cholesteryl) or to supply a cleavable "targetor" moiety that enhances retention on

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the brain side of the barrier (Bodor et al., Science 257:1698-1700, 1992). Alternatively, the polypeptide may be linked to an antibody to the transferrin receptor, in order to exploit that receptor's role in transporting
5 iron across the blood-brain barrier, as taught by Friden et al., Science 259:373-377, 1993. It is expected that an intravenous dosage equivalent to approximately 1 to 100 μ moles of the polypeptide of the invention per kg per day, or an intrathecally administered dosage of
10 approximately 0.1 to 50 μ moles per kg per day, will be effective in blocking activation of G_0 in an Alzheimer's patient. If the polypeptide is sufficiently protected from proteolytic degradation, as described above, it may also be administered orally in appropriately higher
15 doses. Alternatively, the compound may be incorporated into a slow-release implant to ensure a relatively constant supply of the therapeutic to the patient's brain.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Nishimoto, Ikuo

(ii) TITLE OF INVENTION: ALZHEIMER'S DISEASE THERAPEUTICS

(iii) NUMBER OF SEQUENCES: 30

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: Fish & Richardson
(B) STREET: 225 Franklin Street
(C) CITY: Boston
(D) STATE: Massachusetts
(E) COUNTRY: U.S.A.
(F) ZIP: 02110-2804

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: 3.5" Diskette, 1.44 Mb
(B) COMPUTER: IBM PS/2 Model 50Z or 55SX
(C) OPERATING SYSTEM: MS-DOS (Version 5.0)
(D) SOFTWARE: WordPerfect (Version 5.1)

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER: 08/019,208
(B) FILING DATE: February 18, 1993
(C) CLASSIFICATION:

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(A) APPLICATION NUMBER:
(B) FILING DATE:

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: Clark, Paul T.
(B) REGISTRATION NUMBER: 30,162
(C) REFERENCE/DOCKET NUMBER: 00786/154001

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: (617) 542-5070
(B) TELEFAX: (617) 542-8906
(C) TELEX: 200154

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

- 26 -

His His Gly Val Val Glu Val Asp Ala Ala Val Thr Pro Glu Glu Arg
 1 5 10 15

His Leu Ser Lys
 20

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1910
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

TGTGGCAGGG AAGGGGCCAC C ATG GGA TGT ACG CTG AGC GCA GAG GAG AGA	51
Met Gly Cys Thr Leu Ser Ala Glu Glu Arg	
1 5 10	
GCC GCC CTC GAG CGG AGC AAG GCG ATT GAG AAA AAC CTA AAA GAA GAT	99
Ala Ala Leu Glu Arg Ser Lys Ala Ile Glu Lys Asn Leu Lys Glu Asp	
15 20 25	
GGC ATC AGC GCC GCC AAA GAC GTG AAA TTA CTC CTG CTG GGG GCT GGA	147
Gly Ile Ser Ala Ala Lys Asp Val Lys Leu Leu Leu Gly Ala Gly	
30 35 40	
GAA TCA GGA AAA AGC ACC ATT GTG AAG CAG ATG AAG ATC ATC CAT GAA	195
Glu Ser Gly Lys Ser Thr Ile Val Lys Gln Met Lys Ile Ile His Glu	
45 50 55	
GAT GGC TTC TCT GGG GAA GAC GTG AAG CAG TAC AAG CCT GTG GTC TAC	243
Asp Gly Phe Ser Gly Glu Asp Val Lys Gln Tyr Lys Pro Val Val Tyr	
60 65 70	
AGC AAC ACC ATC CAG TCT CTG GCG GCC ATT GTC CGG GCC ATG GAC ACT	291
Ser Asn Thr Ile Gln Ser Leu Ala Ala Ile Val Arg Ala Met Asp Thr	
75 80 85 90	
TTG GGC GTG GAG TAT GGT GAC AAG GAG AGG AAG ACG GAC TCC AAG ATG	339
Leu Gly Val Glu Tyr Gly Asp Lys Glu Arg Lys Thr Asp Ser Lys Met	
95 100 105	
GTG TGT GAC GTG GTG AGT CGT ATG GAA GAC ACT GAA CCG TTC TCT GCA	387
Val Cys Asp Val Val Ser Arg Met Glu Asp Thr Glu Pro Phe Ser Ala	
110 115 120	
GAA CTT CTT TCT GCC ATG ATG CGA CTC TGG GGC GAC TCG GGG ATC CAG	435
Glu Leu Leu Ser Ala Met Met Arg Leu Trp Gly Asp Ser Gly Ile Gln	
125 130 135	
GAG TGC TTC AAC CGA TCT CGG GAG TAT CAG CTC AAT GAC TCT GCC AAA	483
Glu Cys Phe Asn Arg Ser Arg Glu Tyr Gln Leu Asn Asp Ser Ala Lys	
140 145 150	
TAC TAC CTG GAC AGC CTG GAT CGG ATT GGA GCC GGT GAC TAC CAG CCC	531
Tyr Tyr Leu Asp Ser Leu Asp Arg Ile Gly Ala Gly Asp Tyr Gln Pro	
155 160 165 170	

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ACT GAG CAG GAC ATC CTC CGA ACC AGA GTC AAA ACA ACT GGC ATC GTA Thr Glu Gln Asp 175 Leu Arg Thr Arg Val Lys Thr Thr Gly Ile Val 185	579
GAA ACC CAC TTC ACC TTC AAG AAC CTC CAC TTC AGG CTG TTT GAC GTC Glu Thr His Phe Thr Phe Lys Asn Leu His Phe Arg Leu Phe Asp Val 200	627
GGG GGC CAG CGA TCT GAA CGC AAG AAG TGG ATC CAC TGC TTT GAG GAT Gly Gly Gln Arg Ser Glu Arg Lys Lys Trp Ile His Cys Phe Glu Asp 215	675
GTC ACG GCC ATC ATC TTC TGT GTC GCA CTC AGC GGC TAT GAC CAG GTG Val Thr Ala Ile Ile Phe Cys Val Ala Leu Ser Gly Tyr Asp Gln Val 230	723
CTC CAC GAG GAC GAA ACC ACG AAC CGC ATG CAC GAG TCT CTC ATG CTC Leu His Glu Asp Glu Thr Thr Asn Arg Met His Glu Ser Leu Met Leu 250	771
TTC GAC TCC ATC TGT AAC AAC AAG TTT TTC ATT GAT ACC TCC ATC ATC Phe Asp Ser Ile Cys Asn Asn Lys Phe Phe Ile Asp Thr Ser Ile Ile 265	819
CTC TTC CTC AAC AAG AAA GAC CTC TTT GGC GAG AAG ATT AAG AAG TCA Leu Phe Leu Asn Lys Lys Asp Leu Phe Gly Glu Lys Ile Lys Lys Ser 280	867
CCC TTG ACC ATC TGC TTT CCC GAA TAC CCA GGC TCC AAC ACC TAT GAA Pro Leu Thr Ile Cys Phe Pro Glu Tyr Pro Gly Ser Asn Thr Tyr Glu 295	915
GAT GCA GCT GCC TAC ATC CAA ACA CAG TTT GAA AGC AAA AAC CGC TCA Asp Ala Ala Ala Tyr Ile Gln Thr Gln Phe Glu Ser Lys Asn Arg Ser 310	963
CCC AAC AAA GAA ATT TAC TGT CAC ATG ACT TGT GCC ACA GAC ACG AAT Pro Asn Lys Glu Ile Tyr Cys His Met Thr Cys Ala Thr Asp Thr Asn 330	1011
AAT ATC CAG GTG GTA TTC GAC GCC GTC ACC GAC ATC ATC ATT GCC AAC Asn Ile Gln Val Val Phe Asp Ala Val Thr Asp Ile Ile Ile Ala Asn 345	1059
AAT CTC CGG GGC TGC GGC TTG TAC TGACCTCTTG TCCTGTATAG CAACCTATTT Asn Leu Arg Gly Cys Gly Leu Tyr 350	1113
GACTGCTTCA TGGACTCTTT GCTGTTGATG TTGATCTCCT GGTAGCATGA CCTTTGGCCT	1173
TTGTAAGACA CACAGCCTTT CTGTACCAAG CCCCTGTCTA ACCTACGACC CCAGAGTGAC	1233
TGACGGCTGT GTATTTCTGT AGAATGCTGT AGAATACAGT TTTAGTTGAG TCTTTACATT	1293
TAGAACTTGA AAGGATTTTA AAAAACAAAA CAAAAACCAT TTCTCATGTG CTTTGTAGCT	1353
TTAAAAGAAA AAAGGAAAAC TCACCATTTA ATCCATATTT CCTTTTATT TTGAAGTTTA	1413
AAAAAAAAT GTCTGTACCC ACACCCTCCC CCTTCCCCAC CTCAGCAGAA CTGGGGCTGG	1473
CACACAGAGG CAGTGCTGGG CCTGGCGCCT CCCAGGGCTT CTGTGCAGCC CATGGCTGGT	1533
GGGAACATGT CAGGCTAGTC TGTCTAGAAG GCCACTGGCC ACTGTACCCA CCCTTCCCCA	1593

- 28 -

TGCCTGTGGG CTGCCCAGAC ACCTCATATA CCACCAGGCA GTGGCAGCTC CGCCCTGCTC 1653
 AGCCATGCCA CTCCAACAC ACTCAAAGTT TGCCTAGAAA AAGCACAGCT CTGGCAGGGG 1713
 TAGCTGCCAC AGACAACGCT CATCACCTAT AGAAATCCAG CCCTATAGAA GCAATTCACC 1773
 CAGCCCCTTC CTACACTCCC TTTGTGTTGT TAACTTTTTG GTTTTCTGG TCCTAGTGAG 1833
 TGCCTCCCAT GCATACCTGA CCAGCTCTGC CAGTGTCTGG GGTCTGGGGA ACAGGGGTG 1893
 TGTGGTTTGG TTTTGG 1910

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 15
 (B) TYPE: amino acid
 (C) STRANDEDNESS:
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

Asp Ala Val Thr Asp Ile Ile Ile Ala Lys Asn Leu Arg Gly Cys
 1 5 10 15

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 3
 (B) TYPE: amino acid
 (C) STRANDEDNESS:
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Met Gly Cys
 1

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 18
 (B) TYPE: amino acid
 (C) STRANDEDNESS:
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

Ile Glu Lys Asn Leu Lys Glu Asp Gly Ile Ser Ala Ala Lys Asp Val
 1 5 10 15

Lys Leu

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 6:

- 29 -

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 47
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

Lys Lys Lys Gln Tyr Thr Ser Ile His His Gly Val Val Glu Val Asp
1 5 10 15
Ala Ala Val Thr Pro Glu Glu Arg His Leu Ser Lys Met Gln Gln Asn
20 25 30
Gly Tyr Glu Asn Pro Thr Tyr Lys Phe Phe Glu Gln Met Gln Asn
35 40 45

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 10
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

Thr Val Ile Val Ile Thr Leu Val Met Leu
1 5 10

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 24
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

Gly Ala Ile Ile Gly Leu Met Val Gly Gly Val Val Ile Ala Thr Val
1 5 10 15
Ile Val Ile Thr Leu Val Met Leu
20

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 2085
(B) TYPE: nucleic acid
(C) STRANDEDNESS: doubl
(D) TOPOLOGY: linear

- 30 -

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

ATG CTG CCC GGT TTG GCA CTG CTC CTG CTG GCC GCC TGG ACG GCT CGG Met Leu Pro Gly Leu Ala Leu Leu Leu Leu Ala Ala Trp Thr Ala Arg 1 5 10 15	48
GCG CTG GAG GTA CCC ACT GAT GGT AAT GCT GGC CTG CTG GCT GAA CCC Ala Leu Glu Val Pro Thr Asp Gly Asn Ala Gly Leu Leu Ala Glu Pro 20 25 30	96
CAG ATT GCC ATG TTC TGT GGC AGA CTG AAC ATG CAC ATG AAT GTC CAG Gln Ile Ala Met Phe Cys Gly Arg Leu Asn Met His Met Asn Val Gln 35 40 45	144
AAT GGG AAG TGG GAT TCA GAT CCA TCA GGG ACC AAA ACC TGC ATT GAT Asn Gly Lys Trp Asp Ser Asp Pro Ser Gly Thr Lys Thr Cys Ile Asp 50 55 60	192
ACC AAG GAA GGC ATC CTG CAG TAT TGC CAA GAA GTC TAC CCT GGA CTG Thr Lys Glu Gly Ile Leu Gln Tyr Cys Gln Glu Val Tyr Pro Gly Leu 65 70 75 80	240
CAG ATC ACC AAT GTG GTA GAA GCC AAC CAA CCA GTG ACC ATC CAG AAC Gln Ile Thr Asn Val Val Glu Ala Asn Gln Pro Val Thr Ile Gln Asn 85 90 95	288
TGG TGC AAG CGG GGC CGC AAG CAG TGC AAG ACC CAT CCC CAC TTT GTG Trp Cys Lys Arg Gly Arg Lys Gln Cys Lys Thr His Pro His Phe Val 100 105 110	336
ATT CCC TAC CGC TGC TTA GTT GGT GAG TTT GTA AGT GAT GCC CTT CTC Ile Pro Tyr Arg Cys Leu Val Gly Glu Phe Val Ser Asp Ala Leu Leu 115 120 125	384
GTT CCT GAC AAG TGC AAA TTC TTA CAC CAG GAG AGG ATG GAT GTT TGC Val Pro Asp Lys Cys Lys Phe Leu His Gln Glu Arg Met Asp Val Cys 130 135 140	432
GAA ACT CAT CTT CAC TGG CAC ACC GTC GCC AAA GAG ACA TGC AGT GAG Glu Thr His Leu His Trp His Thr Val Ala Lys Glu Thr Cys Ser Glu 145 150 155 160	480
AAG AGT ACC AAC TTG CAT GAC TAC GGC ATG TTG CTG CCC TGC GGA ATT Lys Ser Thr Asn Leu His Asp Tyr Gly Met Leu Leu Pro Cys Gly Ile 165 170 175	528
GAC AAG TTC CGA GGC GTA GAG TTT GTG TGT TGC CCA CTG GCT GAA GAA Asp Lys Phe Arg Gly Val Glu Phe Val Cys Cys Pro Leu Ala Glu Glu 180 185 190	576
AGT GAC AAT GTG GAT TCT GCT GAT GCG GAG GAG GAT GAC TGC GAT GTC Ser Asp Asn Val Asp Ser Ala Asp Ala Glu Glu Asp Asp Cys Asp Val 195 200 205	624
TGG TGG GGC GGA GCA GAC ACA GAC TAT GCA GAT GGG AGT GAA GAC AAA Trp Trp Gly Gly Ala Asp Thr Asp Tyr Ala Asp Gly Ser Glu Asp Lys 210 215 220	672
GTA GTA GAA GTA GCA GAG GAG GAA GAA GTG GCT GAG GTG GAA GAA GAA Val Val Glu Val Ala Glu Glu Glu Val Ala Glu Val Glu Glu Glu 225 230 235 240	720

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GAA GCC GAT GAT GAC GAG GAC GAT GAG GAT GGT GAT GAG GTA GAG GAA Glu Ala Asp Asp Asp Glu Asp Asp Glu Asp Gly Asp Glu Val Glu Glu 245 250 255	768
GAG GCT GAG GAA CCC TAC GAA GAA GCC ACA GAG AGA ACC ACC AGC ATT Glu Ala Glu Glu Pro Tyr Glu Glu Ala Thr Glu Arg Thr Thr Ser Ile 260 265 270	816
GCC ACC ACC ACC ACC ACC ACC ACA GAG TCT GTG GAA GAG GTG GTT CGA Ala Thr Thr Thr Thr Thr Thr Thr Glu Ser Val Glu Glu Val Val Arg 275 280 285	864
GTT CCT ACA ACA GCA GCC AGT ACC CCT GAT GCC GTT GAC AAG TAT CTC Val Pro Thr Thr Ala Ala Ser Thr Pro Asp Ala Val Asp Lys Tyr Leu 290 295 300	912
GAG ACA CCT GGG GAT GAG AAT GAA CAT GCC CAT TTC CAG AAA GCC AAA Glu Thr Pro Gly Asp Glu Asn Glu His Ala His Phe Gln Lys Ala Lys 305 310 315 320	960
GAG AGG CTT GAG GCC AAG CAC CGA GAG AGA ATG TCC CAG GTC ATG AGA Glu Arg Leu Glu Ala Lys His Arg Glu Arg Met Ser Gln Val Met Arg 325 330 335	1008
GAA TGG GAA GAG GCA GAA CGT CAA GCA AAG AAC TTG CCT AAA GCT GAT Glu Trp Glu Glu Ala Glu Arg Gln Ala Lys Asn Leu Pro Lys Ala Asp 340 345 350	1056
AAG AAG GCA GTT ATC CAG CAT TTC CAG GAG AAA GTG GAA TCT TTG GAA Lys Lys Ala Val Ile Gln His Phe Gln Glu Lys Val Glu Ser Leu Glu 355 360 365	1104
CAG GAA GCA GCC AAC GAG AGA CAG CAG CTG GTG GAG ACA CAC ATG GCC Gln Glu Ala Ala Asn Glu Arg Gln Gln Leu Val Glu Thr His Met Ala 370 375 380	1152
AGA GTG GAA GCC ATG CTC AAT GAC CGC CGC CGC CTG GCC CTG GAG AAC Arg Val Glu Ala Met Leu Asn Asp Arg Arg Arg Leu Ala Leu Glu Asn 385 390 395 400	1200
TAC ATC ACC GCT CTG CAG GCT GTT CCT CCT CGG CCT CGT CAC GTG TTC Tyr Ile Thr Ala Leu Gln Ala Val Pro Pro Arg Pro Arg His Val Phe 405 410 415	1248
AAT ATG CTA AAG AAG TAT GTC CGC GCA GAA CAG AAG GAC AGA CAG CAC Asn Met Leu Lys Lys Tyr Val Arg Ala Glu Gln Lys Asp Arg Gln His 420 425 430	1296
ACC CTG AAG CAT TTC GAG CAT GTG CGC ATG GTG GAT CCC AAG AAA GCC Thr Leu Lys His Phe Glu His Val Arg Met Val Asp Pro Lys Lys Ala 435 440 445	1344
GCT CAG ATC CGG TCC CAG GTT ATG ACA CAC CTC CGT GTG ATT TAT GAG Ala Gln Ile Arg Ser Gln Val Met Thr His Leu Arg Val Ile Tyr Glu 450 455 460	1392
CGC ATG AAT CAG TCT CTC TCC CTG CTC TAC AAC GTG CCT GCA GTG GCC Arg Met Asn Gln Ser Leu Ser Leu Leu Tyr Asn Val Pro Ala Val Ala 465 470 475 480	1440
GAG GAG ATT CAG GAT GAA GTT GAT GAG CTG CTT CAG AAA GAG CAA AAC Glu Glu Ile Gln Asp Glu Val Asp Glu Leu Leu Gln Lys Glu Gln Asn 485 490 495	1488

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TAT TCA GAT GAC GTC TTG GCC AAC ATG ATT AGT GAA CCA AGG ATC AGT Tyr Ser Asp Asp Val Leu Ala Asn Met Ile Ser Glu Pro Arg Ile Ser 500 505 510	1536
TAC GGA AAC GAT GCT CTC ATG CCA TCT TTG ACC GAA ACG AAA ACC ACC Tyr Gly Asn Asp Ala Leu Met Pro Ser Leu Thr Glu Thr Lys Thr Thr 515 520 525	1584
GTG GAG CTC CTT CCC GTG AAT GGA GAG TTC AGC CTG GAC GAT CTC CAG Val Glu Leu Leu Pro Val Asn Gly Glu Phe Ser Leu Asp Asp Leu Gln 530 535 540	1632
CCG TGG CAT TCT TTT GGG GCT GAC TCT GTG CCA GCC AAC ACA GAA AAC Pro Trp His Ser Phe Gly Ala Asp Ser Val Pro Ala Asn Thr Glu Asn 545 550 555 560	1680
GAA GTT GAG CCT GTT GAT GCC CGC CCT GCT GCC GAC CGA GGA CTG ACC Glu Val Glu Pro Val Asp Ala Arg Pro Ala Ala Asp Arg Gly Leu Thr 565 570 575	1728
ACT CGA CCA GGT TCT GGG TTG ACA AAT ATC AAG ACG GAG GAG ATC TCT Thr Arg Pro Gly Ser Gly Leu Thr Asn Ile Lys Thr Glu Glu Ile Ser 580 585 590	1776
GAA GTG AAG ATG GAT GCA GAA TTC CGA CAT GAC TCA GGA TAT GAA GTT Glu Val Lys Met Asp Ala Glu Phe Arg His Asp Ser Gly Tyr Glu Val 595 600 605	1824
CAT CAT CAA AAA TTG GTG TTC TTT GCA GAA GAT GTG GGT TCA AAC AAA His His Gln Lys Leu Val Phe Phe Ala Glu Asp Val Gly Ser Asn Lys 610 615 620	1872
GGT GCA ATC ATT GGA CTC ATG GTG GGC GGT GTT GTC ATA GCG ACA GTG Gly Ala Ile Ile Gly Leu Met Val Gly Gly Val Val Ile Ala Thr Val 625 630 635 640	1920
ATC GTC ATC ACC TTG GTG ATG CTG AAG AAG AAA CAG TAC ACA TCC ATT Ile Val Ile Thr Leu Val Met Leu Lys Lys Lys Gln Tyr Thr Ser Ile 645 650 655	1968
CAT CAT GGT GTG GTG GAG GTT GAC GCC GCT GTC ACC CCA GAG GAG CGC His His Gly Val Val Glu Val Asp Ala Ala Val Thr Pro Glu Glu Arg 660 665 670	2016
CAC CTG TCC AAG ATG CAG CAG AAC GGC TAC GAA AAT CCA ACC TAC AAG His Leu Ser Lys Met Gln Gln Asn Gly Tyr Glu Asn Pro Thr Tyr Lys 675 680 685	2064
TTC TTT GAG CAG ATG CAG AAC Phe Phe Glu Gln Met Gln Asn 690 695	2085

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:	16
(B) TYPE:	amino acid
(C) STRANDEDNESS:	
(D) TOPOLOGY:	linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

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Lys Leu Leu Leu Leu Gly Ala Gly Glu Ser Gly Lys Ser Thr Ile Val
 1 5 10 15

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 10
 (B) TYPE: amino acid
 (C) STRANDEDNESS:
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

Met Leu Pro Gly Leu Ala Leu Leu Leu Leu
 1 5 10

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 10
 (B) TYPE: amino acid
 (C) STRANDEDNESS:
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

Asp Ala Glu Phe Arg His Asp Ser Gly Tyr
 1 5 10

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 13:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 19
 (B) TYPE: amino acid
 (C) STRANDEDNESS:
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

Met Gln Gln Asn Gly Tyr Glu Asn Pro Thr Tyr Lys Phe Phe Glu Gln
 1 5 10 15

Met Gln Asn

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 14:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 19
 (B) TYPE: amino acid
 (C) STRANDEDNESS:
 (D) TOPOLOGY: linear

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

His Gly Val Val Glu Val Asp Ala Ala Val Thr Pro Glu Glu Arg His
 1 5 10 15

Leu Ser Lys

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 15:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 18
 (B) TYPE: amino acid
 (C) STRANDEDNESS:
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

Gly Val Val Glu Val Asp Ala Ala Val Thr Pro Glu Glu Arg His Leu
 1 5 10 15

Ser Lys

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 16:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 15
 (B) TYPE: amino acid
 (C) STRANDEDNESS:
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

His His Gly Val Val Glu Val Asp Ala Ala Val Thr Pro Glu Glu
 1 5 10 15

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 17:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 26
 (B) TYPE: amino acid
 (C) STRANDEDNESS:
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

Lys Gln Tyr Thr Ser Ile His His Gly Val Val Glu Val Asp Ala Ala
 1 5 10 15

Val Thr Pro Glu Glu Arg His Leu Ser Lys
 20 25

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 18:

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(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 30
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

Thr Val Ile Val Ile Thr Leu Val Met Leu His His Gly Val Val Glu
1 5 10 15
Val Asp Ala Ala Val Thr Pro Glu Glu Arg His Leu Ser Lys
20 25 30

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 19:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 36
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

CCACGCAGGA TCACGGGATC CATGCTGCCC AGCTTG 36

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 20:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 6
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

GGATCC 6

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 21:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 36
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

CAGTACACAT CCATCTGATG ACATCATGGC GTGGTG 36

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 22:

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(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 35
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

CGCCATCTCT CAGTGATGA ATGCAGCAGA ACGGA 35

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 23:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 656
 (B) TYPE: amino acid
 (C) STRANDEDNESS:
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

Met Leu Pro Gly Leu Ala Leu Leu Leu Leu Ala Ala Trp Thr Ala Arg
 1 5 10 15
 Ala Leu Glu Val Pro Thr Asp Gly Asn Ala Gly Leu Leu Ala Glu Pro
 20 25 30
 Gln Ile Ala Met Phe Cys Gly Arg Leu Asn Met His Met Asn Val Gln
 35 40 45
 Asn Gly Lys Trp Asp Ser Asp Pro Ser Gly Thr Lys Thr Cys Ile Asp
 50 55 60
 Thr Lys Glu Gly Ile Leu Gln Tyr Cys Gln Glu Val Tyr Pro Gly Leu
 65 70 75 80
 Gln Ile Thr Asn Val Val Glu Ala Asn Gln Pro Val Thr Ile Gln Asn
 85 90 95
 Trp Cys Lys Arg Gly Arg Lys Gln Cys Lys Thr His Pro His Phe Val
 100 105 110
 Ile Pro Tyr Arg Cys Leu Val Gly Glu Phe Val Ser Asp Ala Leu Leu
 115 120 125
 Val Pro Asp Lys Cys Lys Phe Leu His Gln Glu Arg Met Asp Val Cys
 130 135 140
 Glu Thr His Leu His Trp His Thr Val Ala Lys Glu Thr Cys Ser Glu
 145 150 155 160
 Lys Ser Thr Asn Leu His Asp Tyr Gly Met Leu Leu Pro Cys Gly Ile
 165 170 175
 Asp Lys Phe Arg Gly Val Glu Phe Val Cys Cys Pro Leu Ala Glu Glu
 180 185 190
 Ser Asp Asn Val Asp Ser Ala Asp Ala Glu Glu Asp Asp Cys Asp Val
 195 200 205

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Trp Trp Gly Gly Ala Asp Thr Asp Tyr Ala Asp Gly Ser Glu Asp Lys
 210 215 220
 Val Val Glu Val Ala Glu Glu Glu Glu Val Ala Glu Val Glu Glu Glu
 225 230 235 240
 Glu Ala Asp Asp Asp Glu Asp Asp Glu Asp Gly Asp Glu Val Glu Glu
 245 250 255
 Glu Ala Glu Glu Pro Tyr Glu Glu Ala Thr Glu Arg Thr Thr Ser Ile
 260 265 270
 Ala Thr Thr Thr Thr Thr Thr Thr Glu Ser Val Glu Glu Val Val Arg
 275 280 285
 Val Pro Thr Thr Ala Ala Ser Thr Pro Asp Ala Val Asp Lys Tyr Leu
 290 295 300
 Glu Thr Pro Gly Asp Glu Asn Glu His Ala His Phe Gln Lys Ala Lys
 305 310 315 320
 Glu Arg Leu Glu Ala Lys His Arg Glu Arg Met Ser Gln Val Met Arg
 325 330 335
 Glu Trp Glu Glu Ala Glu Arg Gln Ala Lys Asn Leu Pro Lys Ala Asp
 340 345 350
 Lys Lys Ala Val Ile Gln His Phe Gln Glu Lys Val Glu Ser Leu Glu
 355 360 365
 Gln Glu Ala Ala Asn Glu Arg Gln Gln Leu Val Glu Thr His Met Ala
 370 375 380
 Arg Val Glu Ala Met Leu Asn Asp Arg Arg Arg Leu Ala Leu Glu Asn
 385 390 395 400
 Tyr Ile Thr Ala Leu Gln Ala Val Pro Pro Arg Pro Arg His Val Phe
 405 410 415
 Asn Met Leu Lys Lys Tyr Val Arg Ala Glu Gln Lys Asp Arg Gln His
 420 425 430
 Thr Leu Lys His Phe Glu His Val Arg Met Val Asp Pro Lys Lys Ala
 435 440 445
 Ala Gln Ile Arg Ser Gln Val Met Thr His Leu Arg Val Ile Tyr Glu
 450 455 460
 Arg Met Asn Gln Ser Leu Ser Leu Leu Tyr Asn Val Pro Ala Val Ala
 465 470 475 480
 Glu Glu Ile Gln Asp Glu Val Asp Glu Leu Leu Gln Lys Glu Gln Asn
 485 490 495
 Tyr Ser Asp Asp Val Leu Ala Asn Met Ile Ser Glu Pro Arg Ile Ser
 500 505 510
 Tyr Gly Asn Asp Ala Leu Met Pro Ser Leu Thr Glu Thr Lys Thr Thr
 515 520 525
 Val Glu Leu Leu Pro Val Asn Gly Glu Phe Ser Leu Asp Asp Leu Gln
 530 535 540

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Pro Trp His Ser Phe Gly Ala Asp Ser Val Pro Ala Asn Thr Glu Asn
 545 550 555 560

Glu Val Glu Pro Val Asp Ala Arg Pro Ala Ala Asp Arg Gly Leu Thr
 565 570 575

Thr Arg Pro Gly Ser Gly Leu Thr Asn Ile Lys Thr Glu Glu Ile Ser
 580 585 590

Glu Val Lys Met Asp Ala Glu Phe Arg His Asp Ser Gly Tyr Glu Val
 595 600 605

His His Gln Lys Leu Val Phe Phe Ala Glu Asp Val Gly Ser Asn Lys
 610 615 620

Gly Ala Ile Ile Gly Leu Met Val Gly Gly Val Val Ile Ala Thr Val
 625 630 635 640

Ile Val Ile Thr Leu Val Met Leu Lys Lys Lys Gln Tyr Thr Ser Ile
 645 650 655

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 24:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 676
 (B) TYPE: amino acid
 (C) STRANDEDNESS:
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

Met Leu Pro Gly Leu Ala Leu Leu Leu Ala Ala Trp Thr Ala Arg
 1 5 10 15

Ala Leu Glu Val Pro Thr Asp Gly Asn Ala Gly Leu Leu Ala Glu Pro
 20 25 30

Gln Ile Ala Met Phe Cys Gly Arg Leu Asn Met His Met Asn Val Gln
 35 40 45

Asn Gly Lys Trp Asp Ser Asp Pro Ser Gly Thr Lys Thr Cys Ile Asp
 50 55 60

Thr Lys Glu Gly Ile Leu Gln Tyr Cys Gln Glu Val Tyr Pro Gly Leu
 65 70 75 80

Gln Ile Thr Asn Val Val Glu Ala Asn Gln Pro Val Thr Ile Gln Asn
 85 90 95

Trp Cys Lys Arg Gly Arg Lys Gln Cys Lys Thr His Pro His Phe Val
 100 105 110

Ile Pro Tyr Arg Cys Leu Val Gly Glu Phe Val Ser Asp Ala Leu Leu
 115 120 125

Val Pro Asp Lys Cys Lys Phe Leu His Gln Glu Arg Met Asp Val Cys
 130 135 140

Glu Thr His Leu His Trp His Thr Val Ala Lys Glu Thr Cys Ser Glu
 145 150 155 160

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Lys Ser Thr Asn Leu His Asp Tyr Gly Met Leu Leu Pro Cys Gly Ile
 165 170 175
 Asp Lys Phe Arg Gly Val Glu Phe Val Cys Cys Pro Leu Ala Glu Glu
 180 185 190
 Ser Asp Asn Val Asp Ser Ala Asp Ala Glu Glu Asp Asp Cys Asp Val
 195 200 205
 Trp Trp Gly Gly Ala Asp Thr Asp Tyr Ala Asp Gly Ser Glu Asp Lys
 210 215 220
 Val Val Glu Val Ala Glu Glu Glu Glu Val Ala Glu Val Glu Glu Glu
 225 230 235 240
 Glu Ala Asp Asp Asp Glu Asp Asp Glu Asp Gly Asp Glu Val Glu Glu
 245 250 255
 Glu Ala Glu Glu Pro Tyr Glu Glu Ala Thr Glu Arg Thr Thr Ser Ile
 260 265 270
 Ala Thr Thr Thr Thr Thr Thr Thr Glu Ser Val Glu Glu Val Val Arg
 275 280 285
 Val Pro Thr Thr Ala Ala Ser Thr Pro Asp Ala Val Asp Lys Tyr Leu
 290 295 300
 Glu Thr Pro Gly Asp Glu Asn Glu His Ala His Phe Gln Lys Ala Lys
 305 310 315 320
 Glu Arg Leu Glu Ala Lys His Arg Glu Arg Met Ser Gln Val Met Arg
 325 330 335
 Glu Trp Glu Glu Ala Glu Arg Gln Ala Lys Asn Leu Pro Lys Ala Asp
 340 345 350
 Lys Lys Ala Val Ile Gln His Phe Gln Glu Lys Val Glu Ser Leu Glu
 355 360 365
 Gln Glu Ala Ala Asn Glu Arg Gln Gln Leu Val Glu Thr His Met Ala
 370 375 380
 Arg Val Glu Ala Met Leu Asn Asp Arg Arg Arg Leu Ala Leu Glu Asn
 385 390 395 400
 Tyr Ile Thr Ala Leu Gln Ala Val Pro Pro Arg Pro Arg His Val Phe
 405 410 415
 Asn Met Leu Lys Lys Tyr Val Arg Ala Glu Gln Lys Asp Arg Gln His
 420 425 430
 Thr Leu Lys His Phe Glu His Val Arg Met Val Asp Pro Lys Lys Ala
 435 440 445
 Ala Gln Ile Arg Ser Gln Val Met Thr His Leu Arg Val Ile Tyr Glu
 450 455 460
 Arg Met Asn Gln Ser Leu Ser Leu Leu Tyr Asn Val Pro Ala Val Ala
 465 470 475 480
 Glu Glu Ile Gln Asp Glu Val Asp Glu Leu Leu Gln Lys Glu Gln Asn
 485 490 495

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Tyr Ser Asp Asp Val Leu Ala Asn Met Ile Ser Glu Pro Arg Ile Ser
 500 505 510
 Tyr Gly Asn Asp Ala Leu Met Pro Ser Leu Thr Glu Thr Lys Thr Thr
 515 520 525
 Val Glu Leu Leu Pro Val Asn Gly Glu Phe Ser Leu Asp Asp Leu Gln
 530 535 540
 Pro Trp His Ser Phe Gly Ala Asp Ser Val Pro Ala Asn Thr Glu Asn
 545 550 555 560
 Glu Val Glu Pro Val Asp Ala Arg Pro Ala Ala Asp Arg Gly Leu Thr
 565 570 575
 Thr Arg Pro Gly Ser Gly Leu Thr Asn Ile Lys Thr Glu Glu Ile Ser
 580 585 590
 Glu Val Lys Met Asp Ala Glu Phe Arg His Asp Ser Gly Tyr Glu Val
 595 600 605
 His His Gln Lys Leu Val Phe Phe Ala Glu Asp Val Gly Ser Asn Lys
 610 615 620
 Gly Ala Ile Ile Gly Leu Met Val Gly Gly Val Val Ile Ala Thr Val
 625 630 635 640
 Ile Val Ile Thr Leu Val Met Leu Lys Lys Lys Gln Tyr Thr Ser Ile
 645 650 655
 His His Gly Val Val Glu Val Asp Ala Ala Val Thr Pro Glu Glu Arg
 660 665 670
 His Leu Ser Lys
 675

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 25:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 58
 (B) TYPE: amino acid
 (C) STRANDEDNESS:
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

Ala Asp Ser Val Pro Ala Asn Thr Glu Asn Glu Val Glu Pro Val Asp
 1 5 10 15
 Ala Arg Pro Ala Ala Asp Arg Gly Leu Thr Thr Arg Pro Gly Ser Gly
 20 25 30
 Leu Thr Asn Ile Lys Thr Glu Glu Ile Ser Glu Val Lys Met Asp Ala
 35 40 45
 Glu Phe Arg His Asp Ser Gly Tyr Glu Val
 50 55

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(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 26:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 56
 (B) TYPE: amino acid
 (C) STRANDEDNESS:
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

Val Ile Val Ile Thr Leu Val Met Leu Lys Lys Lys Gln Tyr Thr Ser
 1 5 10 15
 Ile His His Gly Val Val Glu Val Asp Ala Ala Val Thr Pro Glu Glu
 20 25 30
 Arg His Leu Ser Lys Met Gln Gln Asn Gly Tyr Glu Asn Pro Thr Tyr
 35 40 45
 Lys Phe Phe Glu Gln Met Gln Asn
 50 55

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 27:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 695
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

Met Leu Pro Gly Leu Ala Leu Leu Leu Leu Ala Ala Trp Thr Ala Arg
 1 5 10 15
 Ala Leu Glu Val Pro Thr Asp Gly Asn Ala Gly Leu Leu Ala Glu Pro
 20 25 30
 Gln Ile Ala Met Phe Cys Gly Arg Leu Asn Met His Met Asn Val Gln
 35 40 45
 Asn Gly Lys Trp Asp Ser Asp Pro Ser Gly Thr Lys Thr Cys Ile Asp
 50 55 60
 Thr Lys Glu Gly Ile Leu Gln Tyr Cys Gln Glu Val Tyr Pro Gly Leu
 65 70 75 80
 Gln Ile Thr Asn Val Val Glu Ala Asn Gln Pro Val Thr Ile Gln Asn
 85 90 95
 Trp Cys Lys Arg Gly Arg Lys Gln Cys Lys Thr His Pro His Phe Val
 100 105 110
 Ile Pro Tyr Arg Cys Leu Val Gly Glu Phe Val Ser Asp Ala Leu Leu
 115 120 125

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Val Pro Asp Lys Cys Lys Phe Leu His Gln Glu Arg Met Asp Val Cys
 130 135 140
 Glu Thr His Leu His Trp His Thr Val Ala Lys Glu Thr Cys Ser Glu
 145 150 155 160
 Lys Ser Thr Asn Leu His Asp Tyr Gly Met Leu Leu Pro Cys Gly Ile
 165 170 175
 Asp Lys Phe Arg Gly Val Glu Phe Val Cys Cys Pro Leu Ala Glu Glu
 180 185 190
 Ser Asp Asn Val Asp Ser Ala Asp Ala Glu Glu Asp Asp Cys Asp Val
 195 200 205
 Trp Trp Gly Gly Ala Asp Thr Asp Tyr Ala Asp Gly Ser Glu Asp Lys
 210 215 220
 Val Val Glu Val Ala Glu Glu Glu Glu Val Ala Glu Val Glu Glu Glu
 225 230 235 240
 Glu Ala Asp Asp Asp Glu Asp Asp Glu Asp Gly Asp Glu Val Glu Glu
 245 250 255
 Glu Ala Glu Glu Pro Tyr Glu Glu Ala Thr Glu Arg Thr Thr Ser Ile
 260 265 270
 Ala Thr Thr Thr Thr Thr Thr Thr Glu Ser Val Glu Glu Val Val Arg
 275 280 285
 Val Pro Thr Thr Ala Ala Ser Thr Pro Asp Ala Val Asp Lys Tyr Leu
 290 295 300
 Glu Thr Pro Gly Asp Glu Asn Glu His Ala His Phe Gln Lys Ala Lys
 305 310 315 320
 Glu Arg Leu Glu Ala Lys His Arg Glu Arg Met Ser Gln Val Met Arg
 325 330 335
 Glu Trp Glu Glu Ala Glu Arg Gln Ala Lys Asn Leu Pro Lys Ala Asp
 340 345 350
 Lys Lys Ala Val Ile Gln His Phe Gln Glu Lys Val Glu Ser Leu Glu
 355 360 365
 Gln Glu Ala Ala Asn Glu Arg Gln Gln Leu Val Glu Thr His Met Ala
 370 375 380
 Arg Val Glu Ala Met Leu Asn Asp Arg Arg Arg Leu Ala Leu Glu Asn
 385 390 395 400
 Tyr Ile Thr Ala Leu Gln Ala Val Pro Pro Arg Pro Arg His Val Phe
 405 410 415
 Asn Met Leu Lys Lys Tyr Val Arg Ala Glu Gln Lys Asp Arg Gln His
 420 425 430
 Thr Leu Lys His Phe Glu His Val Arg Met Val Asp Pro Lys Lys Ala
 435 440 445
 Ala Gln Ile Arg Ser Gln Val Met Thr His Leu Arg Val Ile Tyr Glu
 450 455 460

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Arg Met Asn Gln Ser Leu Ser Leu Leu Tyr Asn Val Pro Ala Val Ala
 465 470 475 480
 Glu Glu Ile Gln Asp Glu Val Asp Glu Leu Leu Gln Lys Glu Gln Asn
 485 490 495
 Tyr Ser Asp Asp Val Leu Ala Asn Met Ile Ser Glu Pro Arg Ile Ser
 500 505 510
 Tyr Gly Asn Asp Ala Leu Met Pro Ser Leu Thr Glu Thr Lys Thr Thr
 515 520 525
 Val Glu Leu Leu Pro Val Asn Gly Glu Phe Ser Leu Asp Asp Leu Gln
 530 535 540
 Pro Trp His Ser Phe Gly Ala Asp Ser Val Pro Ala Asn Thr Glu Asn
 545 550 555 560
 Glu Val Glu Pro Val Asp Ala Arg Pro Ala Ala Asp Arg Gly Leu Thr
 565 570 575
 Thr Arg Pro Gly Ser Gly Leu Thr Asn Ile Lys Thr Glu Glu Ile Ser
 580 585 590
 Glu Val Lys Met Asp Ala Glu Phe Arg His Asp Ser Gly Tyr Glu Val
 595 600 605
 His His Gln Lys Leu Val Phe Phe Ala Glu Asp Val Gly Ser Asn Lys
 610 615 620
 Gly Ala Ile Ile Gly Leu Met Val Gly Gly Val Val Ile Ala Thr Val
 625 630 635 640
 Ile Val Ile Thr Leu Val Met Leu Lys Lys Lys Gln Tyr Thr Ser Ile
 645 650 655
 His His Gly Val Val Glu Val Asp Ala Ala Val Thr Pro Glu Glu Arg
 660 665 670
 His Leu Ser Lys Met Gln Gln Asn Gly Tyr Glu Asn Pro Thr Tyr Lys
 675 680 685
 Phe Phe Glu Gln Met Gln Asn
 690 695

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 28:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 2274
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

GCTGTGGCAG GGAAGGGGCC ACC ATG GGA TGT ACG CTG AGC GCA GAG GAG 50
 Met Gly Cys Thr Leu Ser Ala Glu Glu
 1 5
 AGA GCC GCC CTC GAG CGG AGC AAG GCG ATT GAG AAA AAC CTC AAA GAA 98
 Arg Ala Ala Leu Glu Arg Ser Lys Ala Ile lu Lys Asn Leu Lys Glu
 10 15 20 25

- 44 -

GAT GGC ATC AGC GCC GCC AAA GAC GTG AAA TTA CTC CTG CTG GGG GCT	146
Asp Gly Ile Ser Ala Ala Lys Asp Val Lys Leu Leu Leu Leu Gly Ala	
30 35 40	
GGA GAA TCA GGA AAA AGC ACC ATT GTG AAG CAG ATG AAG ATC ATC CAT	194
Gly Glu Ser Gly Lys Ser Thr Ile Val Lys Gln Met Lys Ile Ile His	
45 50 55	
GAA GAT GGC TTC TCT GGG GAA GAC GTG AAG CAG TAC AAG CCT GTG GTC	242
Glu Asp Gly Phe Ser Gly Glu Asp Val Lys Gln Tyr Lys Pro Val Val	
60 65 70	
TAC AGC AAC ACC ATC CAG TCT CTG GCG GCC ATT GTC CGG GCC ATG GAC	290
Tyr Ser Asn Thr Ile Gln Ser Leu Ala Ala Ile Val Arg Ala Met Asp	
75 80 85	
ACT TTG GGC GTG GAG TAT GGT GAC AAG GAG AGG AAG ACG GAC TCC AAG	338
Thr Leu Gly Val Glu Tyr Gly Asp Lys Glu Arg Lys Thr Asp Ser Lys	
90 95 100 105	
ATG GTG TGT GAC GTG GTG AGT CGT ATG GAA GAC ACT GAA CCG TTC TCT	386
Met Val Cys Asp Val Val Ser Arg Met Glu Asp Thr Glu Pro Phe Ser	
110 115 120	
GCA GAA CTT CTT TCT GCC ATG ATG CGA CTC TGG GGC GAC TCG GGG ATC	434
Ala Glu Leu Ser Ala Met Met Arg Leu Trp Gly Asp Ser Gly Ile	
125 130 135	
CAG GAG TGC TTC AAC CGA TCT CGG GAG TAT CAG CTC AAT GAC TCT GCC	482
Gln Glu Cys Phe Asn Arg Ser Arg Glu Tyr Gln Leu Asn Asp Ser Ala	
140 145 150	
AAA TAC TAC CTG GAC AGC CTG GAT CGG ATT GGA GCC GGT GAC TAC CAG	530
Lys Tyr Tyr Leu Asp Ser Leu Asp Arg Ile Gly Ala Gly Asp Tyr Gln	
155 160 165	
CCC ACT GAG CAG GAC ATC CTC CGA ACC AGA GTC AAA ACA ACT GGC ATC	578
Pro Thr Glu Gln Asp Ile Leu Arg Thr Arg Val Lys Thr Thr Gly Ile	
170 175 180 185	
GTA GAA ACC CAC TTC ACC TTC AAG AAC CTC CAC TTC AGG CTG TTT GAC	626
Val Glu Thr His Phe Thr Phe Lys Asn Leu His Phe Arg Leu Phe Asp	
190 195 200	
GTC GGG GGC CAG CGA TCT GAA CGC AAG AAG TGG ATC CAC TGC TTT GAG	674
Val Gly Gly Gln Arg Ser Glu Arg Lys Lys Trp Ile His Cys Phe Glu	
205 210 215	
GAT GTC ACG GCC ATC ATC TTC TGT GTC GCA CTC AGC GGC TAT GAC CAG	722
Asp Val Thr Ala Ile Ile Phe Cys Val Ala Leu Ser Gly Tyr Asp Gln	
220 225 230	
GTG CTC CAC GAG GAC GAA ACC ACG AAC CGC ATG CAC GAA TCC CTG AAG	770
Val Leu His Glu Asp Glu Thr Thr Asn Arg Met His Glu Ser Leu Lys	
235 240 245	
CTC TTC GAC AGC ATC TGC AAC AAC AAG TGG TTC ACA GAC ACA TCT ATT	818
Leu Phe Asp Ser Ile Cys Asn Asn Lys Trp Phe Thr Asp Thr Ser Ile	
250 255 260 265	
ATC CTG TTT CTC AAC AAG AAG GAC ATA TTT GAG GAG AAG ATC AAG AAG	866
Ile Leu Ph Leu Asn Lys Lys Asp Ile Phe Glu Glu Lys Ile Lys Lys	
270 275 280	

- 45 -

TCC CCA CTC ACC ATC TGC TTT CCT GAA TAC ACA CGC CCC AGT GCC TTC	914
Ser Pro Leu Thr Ile Cys Phe Pro Glu Tyr Thr Gly Pro Ser Ala Phe	
285 290 295	
ACA GAA GCT GTG GCT CAC ATC CAA GGG CAG TAT GAG AGT AAG AAT AAG	962
Thr Glu Ala Val Ala His Ile Gln Gly Gln Tyr Glu Ser Lys Asn Lys	
300 305 310	
TCA GCT CAC AAG GAA GTC TAC AGC CAT GTC ACC TGT GCC ACG GAC ACC	1010
Ser Ala His Lys Glu Val Tyr Ser His Val Thr Cys Ala Thr Asp Thr	
315 320 325	
AAC AAC ATC CAA TTC GTC TTT GAT GCC GTG ACA GAT GTC ATC ATC GCC	1058
Asn Asn Ile Gln Phe Val Phe Asp Ala Val Thr Asp Val Ile Ile Ala	
330 335 340 345	
AAA AAC CTA CGG GGC TGT GGA CTC TAC TGAGCCCTGG CCTCCTACCC	1105
Lys Asn Leu Arg Gly Cys Gly Leu Tyr	
350	
AGCCTGCCAC TCACTCCTCC CCTGGACCCA GAGCTCTGTC ACTGCTCAGA TGCCCTGTTA	1165
ACTGAAGAAA ACCTGGAGGC TAGCCTTGGG GGCAGGAGGA GGCATCCTTT GAGCATCCCC	1225
ACCCACCCA ACTTCAGCCT CGTGACACGT GGAACAGGG TTGGGCAGAG GTGTGGAACA	1285
GCACAAGGCC AGAGACCACG GCATGCCACT TGGGTGCTGC TCACTGGTCA GCTGTGTGTC	1345
TTACACAGAG GCCGAGTGGG CAACACTGCC ATCTGATTCA GAATGGGCAT GCCCTGTCCT	1405
CTGTACCTCT TGTTCAGTGT CCTGGTTTCT CTTCACCTT GGTGATAGGA TGGCTGGCAG	1465
GAAGGCCCA TGGAAGGTGC TGCTTGATTA GGGGATAGTC GATGGCATCT CTCAGCAGTC	1525
CTCAGGGTCT GTTTGGTAGA GGGTGGTTTC GTCGACAAA GCCAACATGG AATCAGGCCA	1585
CTTTTGGGGC GCAAAGACTC AGACTTTGGG GACGGGTTC CTCCTCCTTC ACTTTGGATC	1645
TTGGCCCCTC TCTGGTCATC TTCCCTTGCC CTTGGGCTCC CCAGGATACT CAGCCCTGAC	1705
TCCCATGGGG TTGGGAATAT TCCTTAAGAC TGGCTGACTG CAAAGGTCAC CGATGGAGAA	1765
ACATCCCTGT GCTACAGAAT TGGGGGTGGG ACAGCTGAGG GGGCAGGCGG CTCTTCTCTG	1825
ATAGTTGATG ACAAGCCCTG AGAATGCCAT CTGCTGGCTC CACTCACACG GGCTCAACTG	1885
TCCTGGGTGA TAGTGACTTG CCAGGCCACA GGCTGCAGGT CACAGACAGA GCAGGCAAGC	1945
AGCCTTGCAA CTGCAGATTA CTTAGGGAGA AGCATCCTAG CCCCAGCTAA CTTTGGACAG	2005
TCAGCATATG TCCCTGCCAT CCCTAGACAT CTCCAGTCAG CTGGTATCAC AGCCAGTGGT	2065
TCAGACAGGT TTGAATGCTC ATGTGGCAGG GGGCCCGGTA CCCAGCTTTT GTTCCCTTTA	2125
GTGAGGGTTA ATTGCGCGCT TGGGCTAATC ATGGTCATAG CTGTTGGGCG TTGCTGGCGT	2185
TTTTCCATAG GCTCCGCCCC CTGACGAGAT CACAAAATC GACGCTCAAG TCAGAGGTGG	2245
CGAAACCGAC AGACTATAAG ATACCAGGC	2274

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(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 29:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 18
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 29:

Asp Val Gly Gly Gln Arg Ser Glu Arg Lys Lys Trp Ile His Cys Phe
1 5 10 15
Glu Asp

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 30:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 12
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 30:

Thr Ser Ile Ile Leu Phe Leu Asn Lys Lys Asp Leu
1 5 10

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CLAIMS

1. A method of identifying a therapeutic useful for treating or preventing the symptoms of Alzheimer's disease, which method includes the steps of
 - 5 contacting (a) a first molecule comprising the couplone portion (SEQ ID NO: 1) of amyloid precursor protein (APP) with (b) a second molecule comprising an APP-associating region of G_o (SEQ ID NOS: 3, 4, or 5), in the presence of a candidate compound; and
 - 10 determining whether said candidate compound interferes with the association of said first and second molecules, said interference being an indication that said candidate compound is a therapeutic useful for treating Alzheimer's disease.
- 15 2. The method of claim 1, wherein said determining step is accomplished by
 - immunoprecipitating said first molecule with an antibody specific for APP; and
 - 20 detecting the presence or amount of said second molecule which co-precipitates with said first molecule.
- 25 3. The method of claim 1, wherein said determining step is accomplished by
 - immunoprecipitating said second molecule with an antibody specific for G_o ; and
 - detecting the presence or amount of said first molecule which co-precipitates with said second molecule.
4. The method of claim 1, wherein said first molecule comprises the portion of APP₆₉₅ from residues 649 to 695 (SEQ ID NO: 6).

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5. The method of claim 1, wherein said first molecule comprises the portion of APP₆₉₅ from residues 639 to 648 (SEQ ID NO: 7).

6. The method of claim 1, wherein said first molecule comprises the portion of APP₆₉₅ from residues 640 to 695 (SEQ ID NO: 26).

7. The method of claim 6, wherein said first molecule comprises essentially all of APP₆₉₅ (SEQ ID NO: 27).

8. The method of claim 1, wherein said second molecule comprises the GTP-binding region of G_o (SEQ ID NO: 10).

9. The method of claim 8, wherein said second molecule comprises essentially all of G_o (SEQ ID NO: 2).

10. A method of assaying for a therapeutic useful for treating Alzheimer's disease, which method includes the steps of

contacting (a) a first molecule comprising the couplone region of APP (SEQ ID NO: 1) with (b) a second molecule comprising an APP-associating region of G_o (SEQ ID NO: 3, 4, or 5), in the presence of a candidate compound; and

determining whether said candidate compound interferes with the activation of said second molecule by said first molecule, said interference being an indication that said candidate compound is a therapeutic useful for treating Alzheimer's disease.

11. The method of claim 10, wherein said determining step is accomplished by

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contacting said second molecule with a substrat
comprising GTP or an analog of GTP; and

detecting or measuring the binding of said
substrate to said second molecule, wherein said binding
5 is evidence of said activation of said second molecule by
said first molecule.

12. The method of claim 1, wherein said
contacting step is carried out at a Mg^{2+} concentration
between 1×10^{-7} and 1×10^{-2} M.

10 13. The method of claim 10, wherein said
contacting step is carried out at a Mg^{2+} concentration
between 1×10^{-7} and 1×10^{-2} M.

14. The method of claim 1, wherein said
contacting step is carried out in a cell-free system.

15 15. The method of claim 10, wherein said
contacting step is carried out in a cell-free system.

16. A system for screening candidate Alzheimer's
disease therapeutics, which system comprises
a first polypeptide comprising a sequence
20 essentially identical to that of peptide 20 (SEQ ID
NO: 1);

a second polypeptide comprising a sequence
essentially identical to the anticouplone sequence of G_o
(SEQ ID NO: 3); and

25 a means for detecting either (a) the association
of said first polypeptide with said second polypeptide,
or (b) the activation of said second polypeptide by said
first polypeptide.

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17. A cell-free system for screening candidate Alzheimer's disease therapeutics, which system comprises a first polypeptide comprising a sequence essentially identical to that of peptide 20 (SEQ ID

5 NO: 1); and

a second polypeptide comprising a sequence essentially identical to the anticouplone sequence of G_o (SEQ ID NO: 3).

18. The system of claim 17, wherein said first
10 polypeptide is anchored to a solid material or is in a phospholipid vesicle.

19. The system of claim 17, wherein said second polypeptide further comprises residues 1 to 3 (SEQ ID NO: 4) and 19 to 36 (SEQ ID NO: 5) of G_o .

15 20. The system of claim 19, wherein said second polypeptide comprises G_{o1} or G_{o2} .

21. A method for diminishing the activation of G_o in a neuronal cell by treating the cell with a compound which blocks association of G_o with the cytoplasmic tail
20 of APP.

22. The method of claim 21, wherein the compound is a peptide fragment of G_o or of the cytoplasmic tail of APP.

23. The method of claim 21, wherein said cell is
25 within an animal.

24. The method of claim 23, wherein said animal is a human.

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25. A method for preventing or treating Alzheimer's disease in a patient, comprising treating the patient with a compound which blocks association of G_o with the cytoplasmic tail of APP.

5 26. A method for preventing or treating Alzheimer's disease in a patient, comprising treating the patient with a compound which inhibits activation of neuronal G_o by the cytoplasmic tail of APP.

27. A peptide having less than 50 amino acids and
10 comprising the sequence of peptide 20 (SEQ ID NO: 1).

28. A therapeutic composition comprising the peptide of claim 27 and a pharmaceutically acceptable carrier.

29. A method for identifying a ligand for which
15 APP is a receptor, which method includes the steps of providing an APP molecule and a G_o molecule; contacting a candidate compound with the extracellular domain of said APP molecule, the cytoplasmic tail of said APP molecule being accessible to
20 said G_o molecule, and detecting either (a) association of said G_o molecule with said APP molecule, or (b) activation of said G_o molecule by said APP molecule, said association or activation being evidence that said candidate compound
25 is a ligand of APP.

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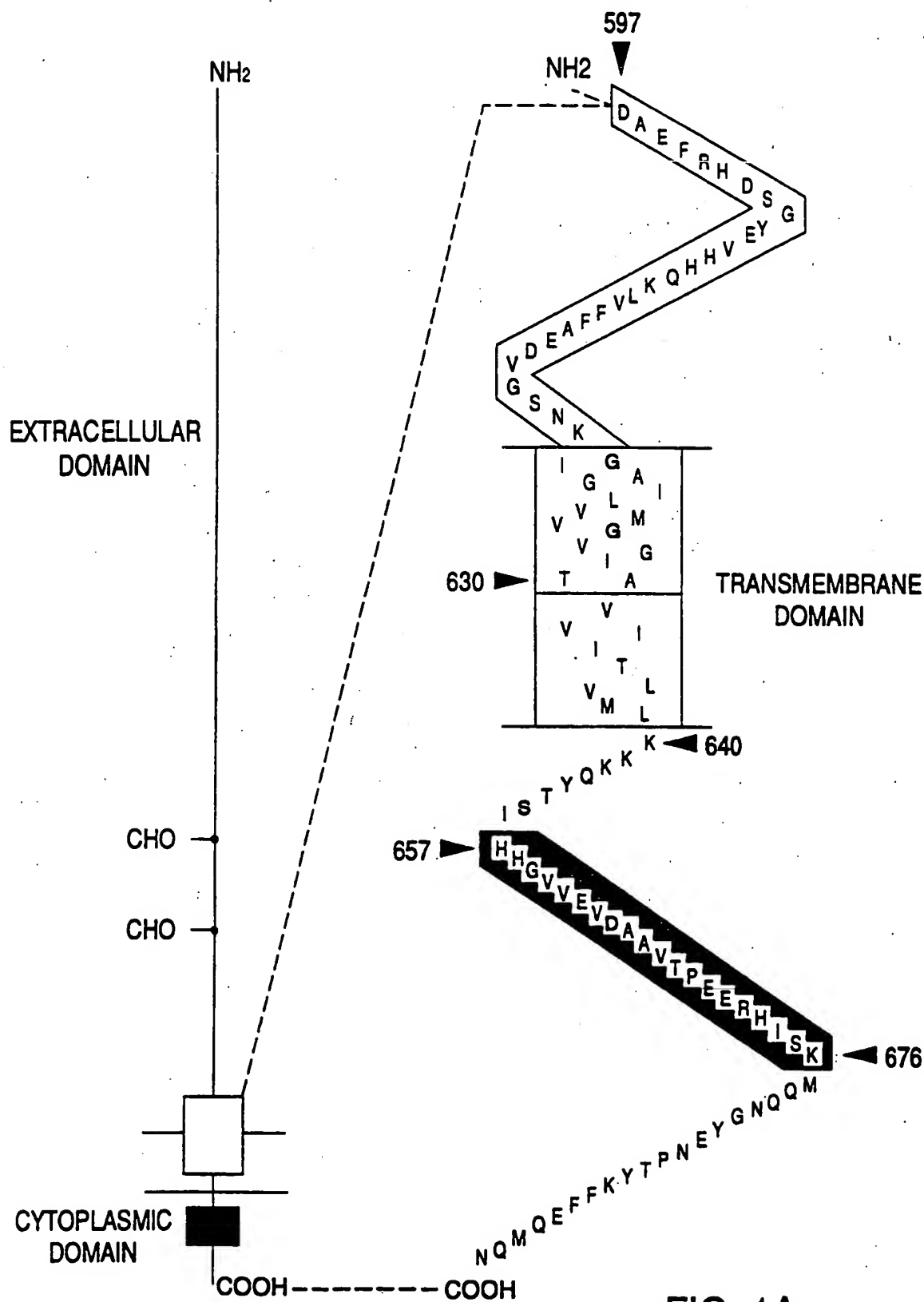


FIG. 1A

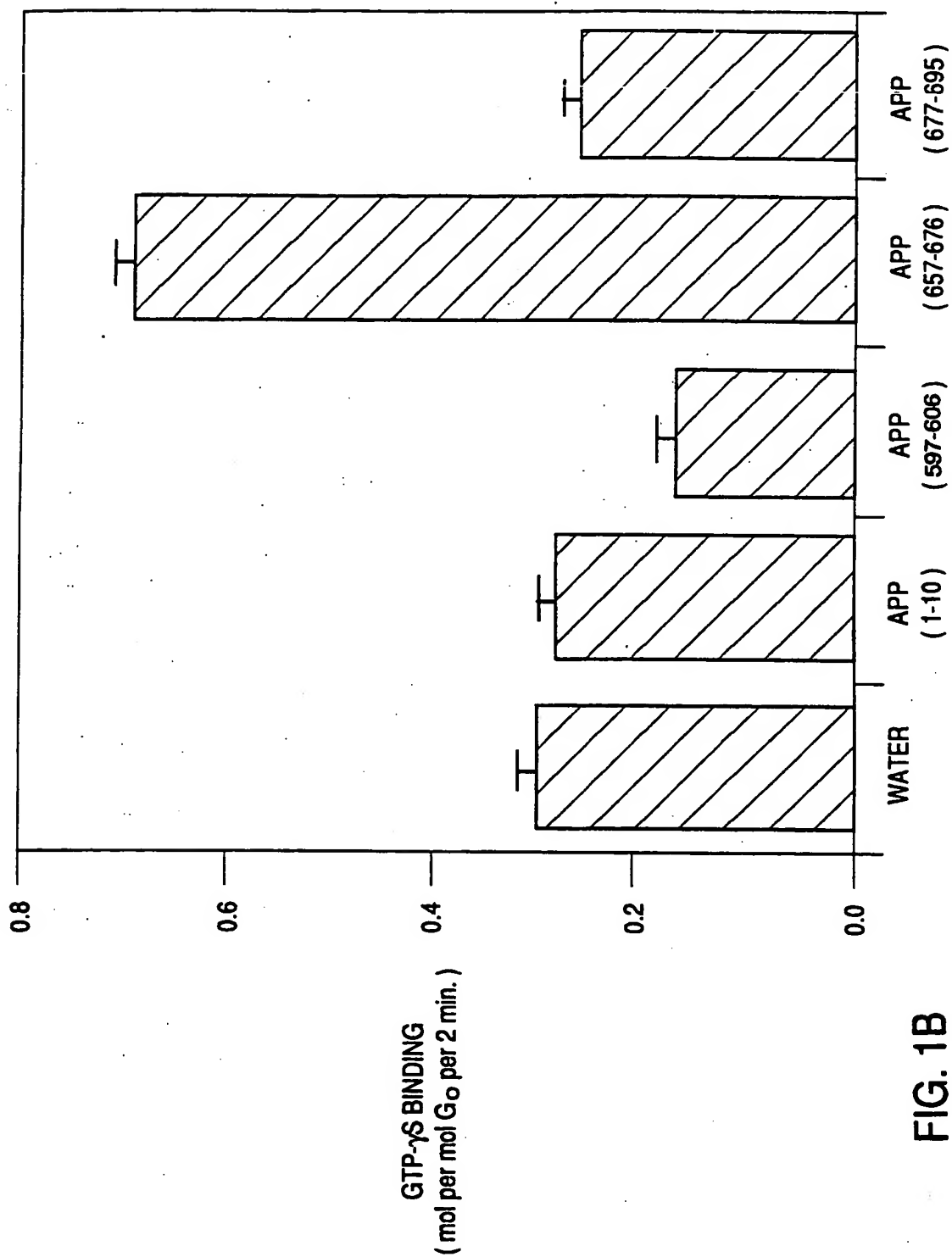


FIG. 1B

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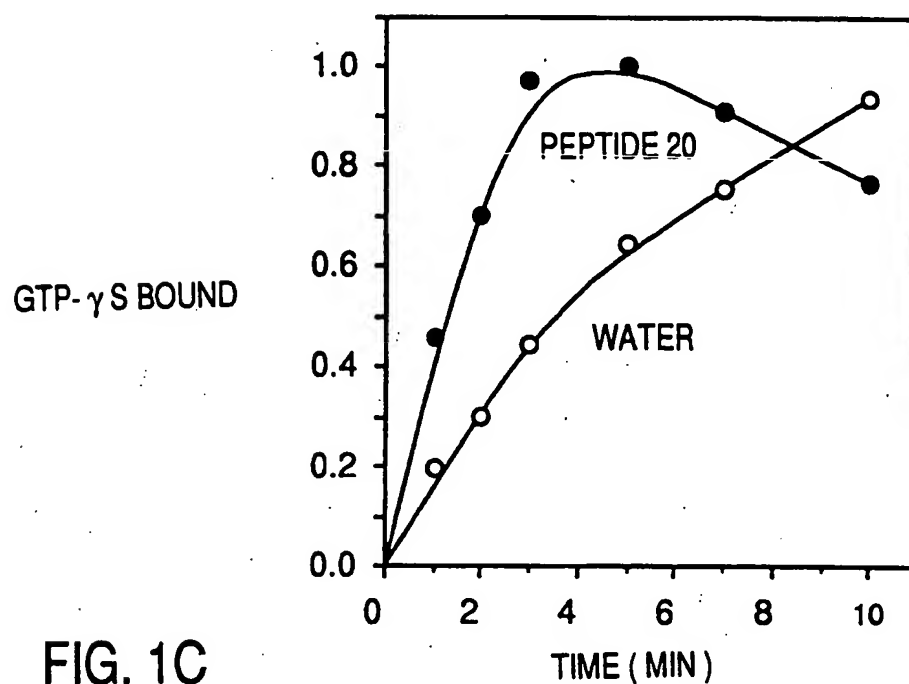


FIG. 1C

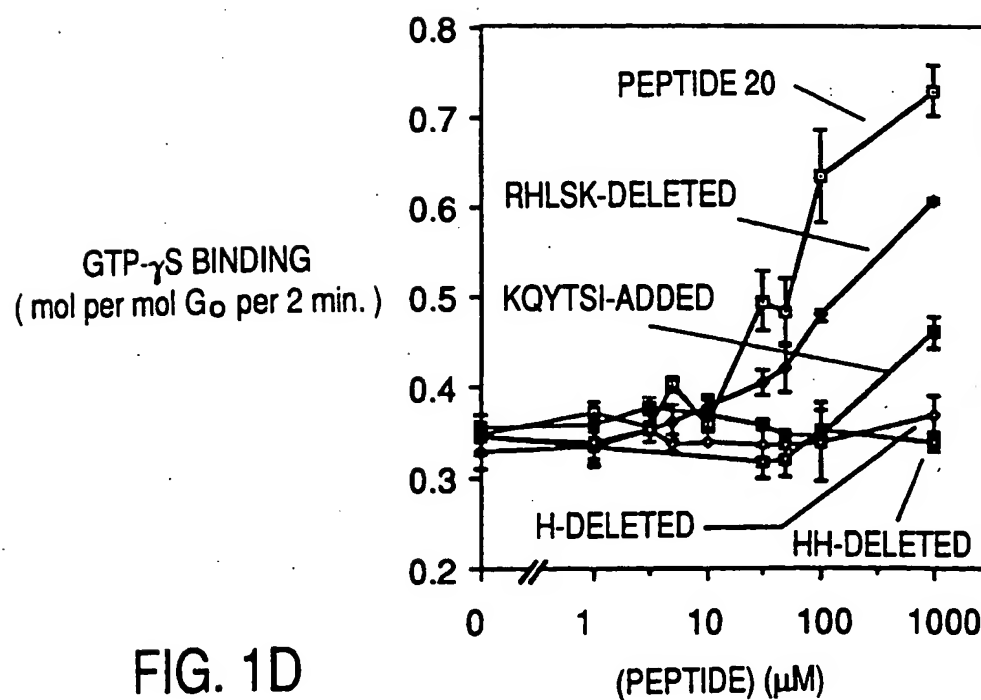


FIG. 1D

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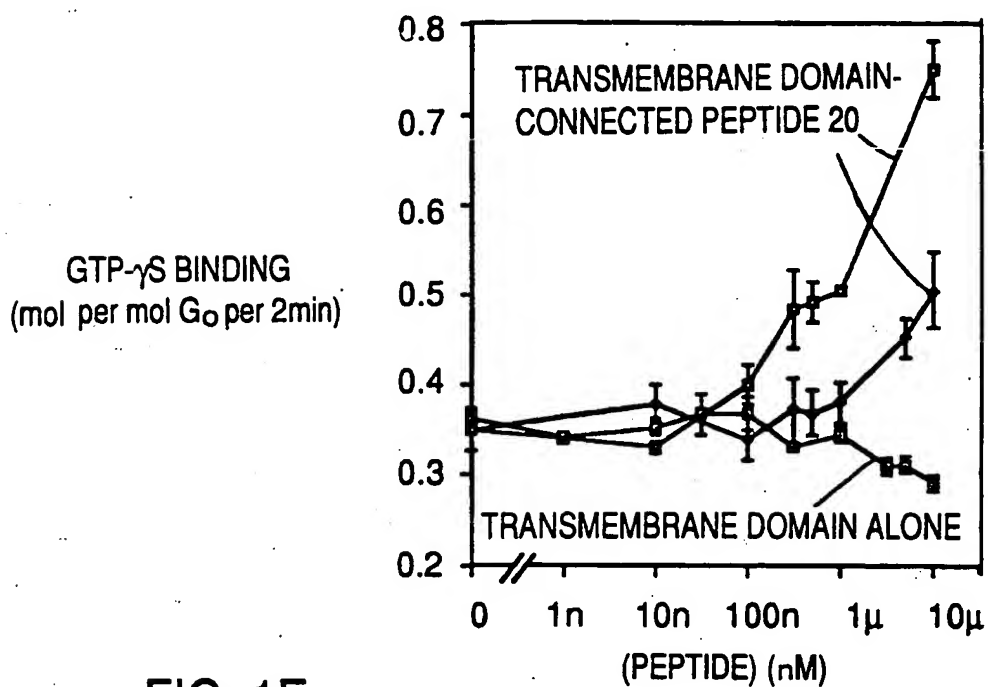


FIG. 1E

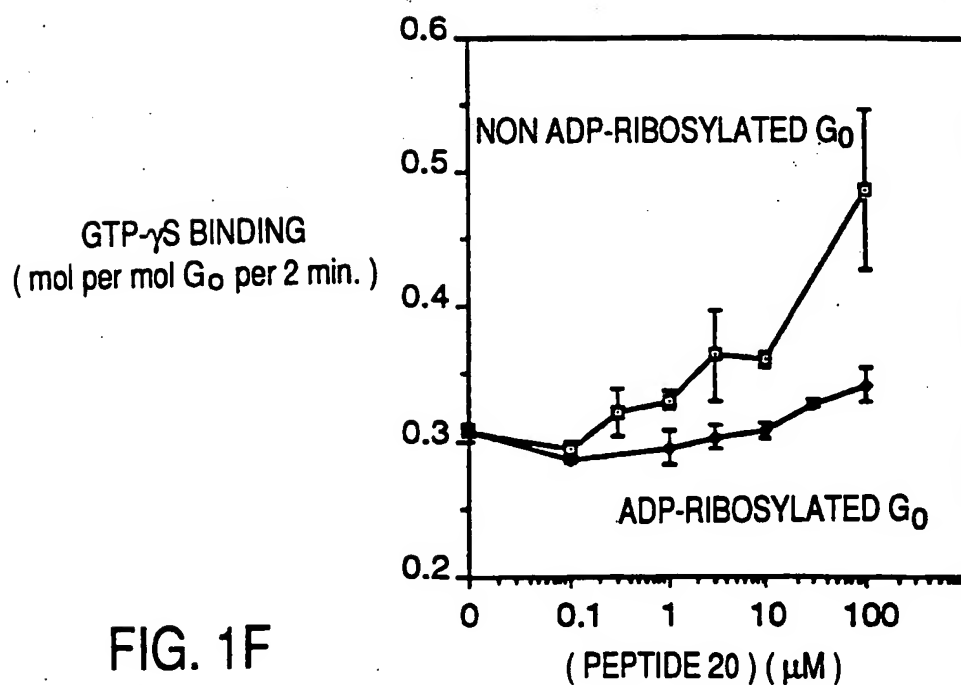


FIG. 1F

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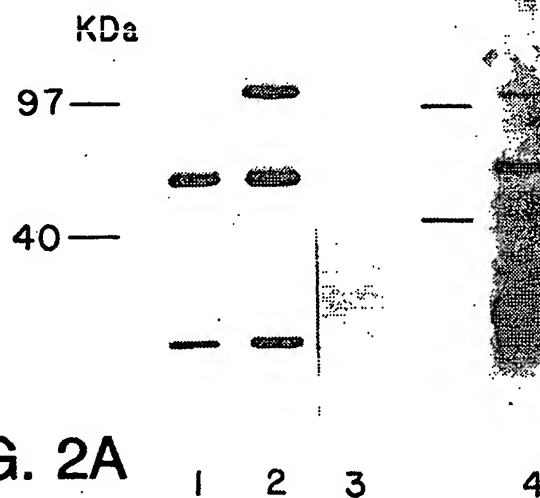


FIG. 2A

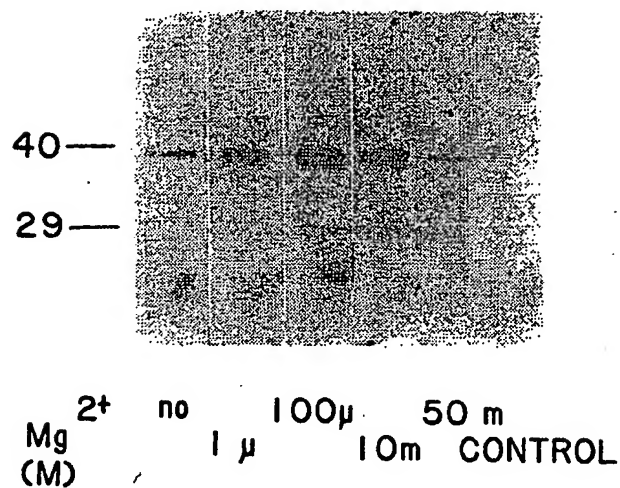


FIG. 2C

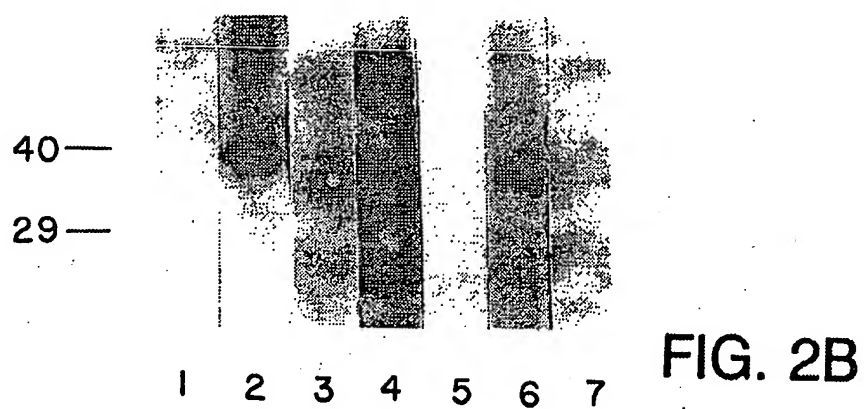


FIG. 2B

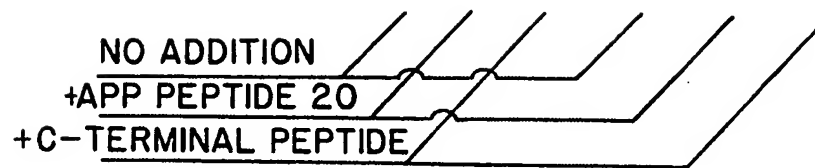
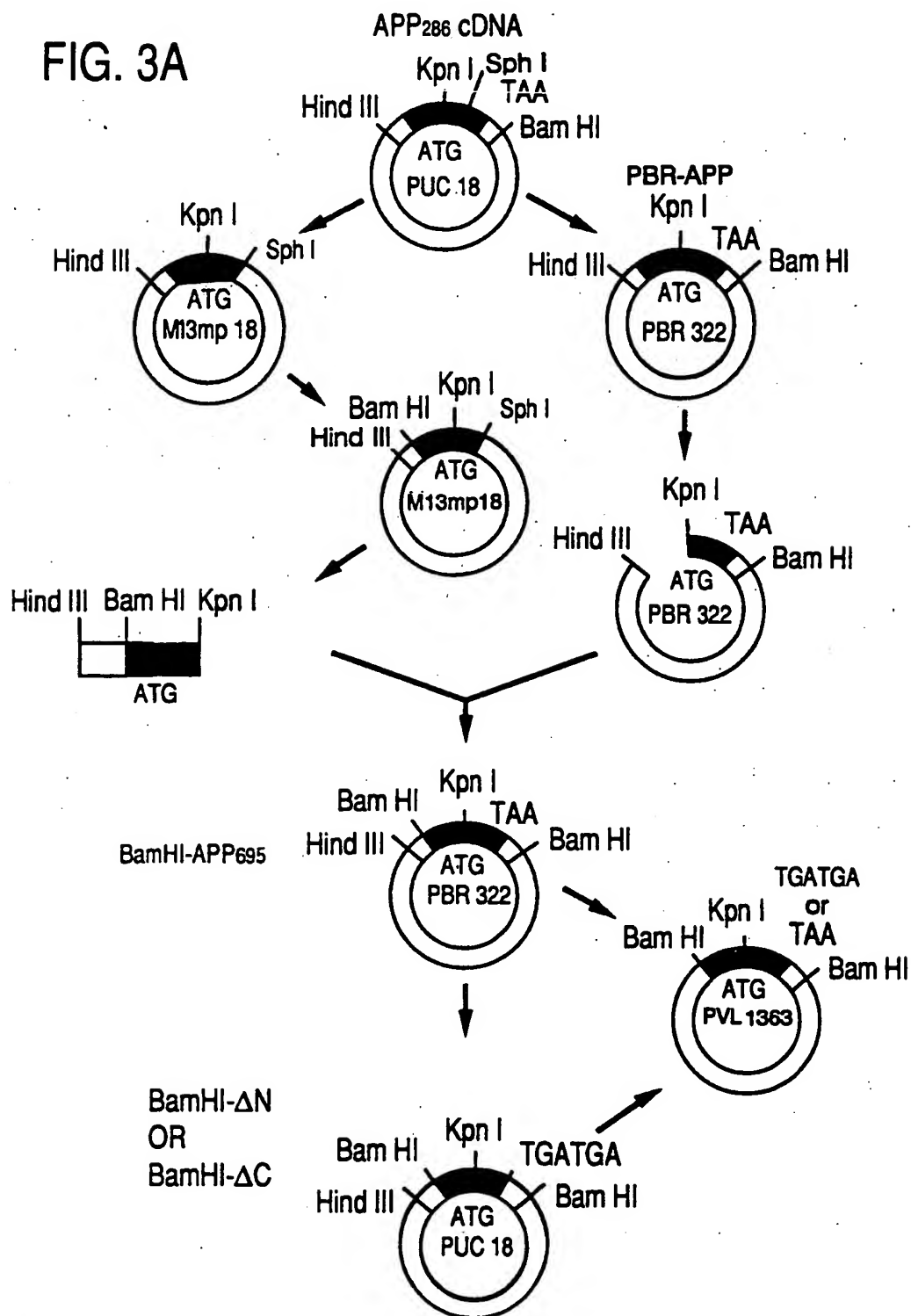


FIG. 2D

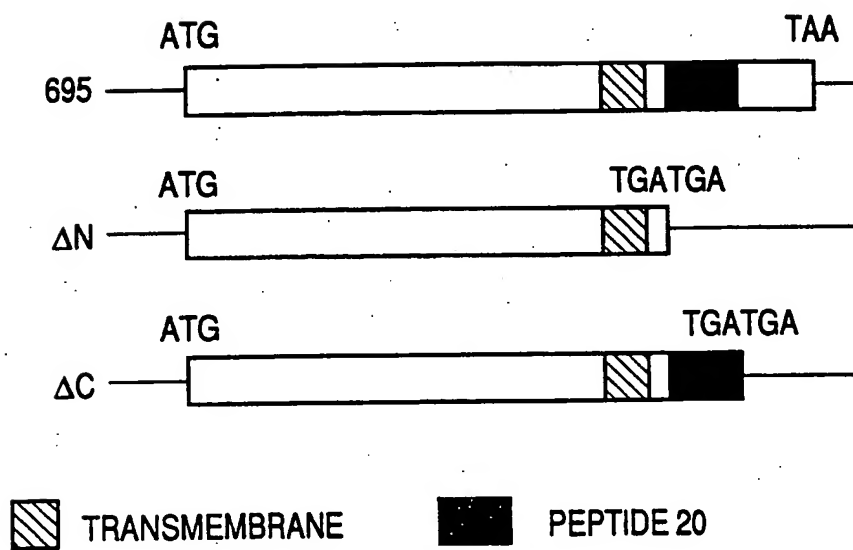
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FIG. 3A



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FIG. 3B



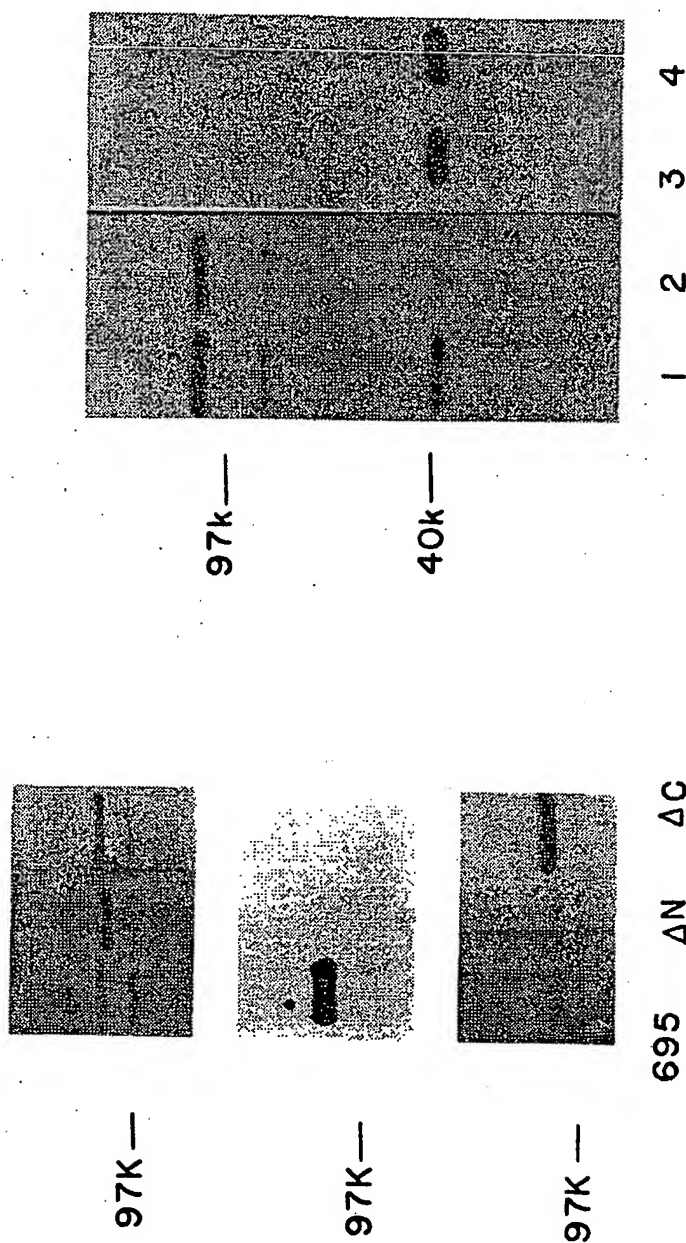


FIG. 3E

FIG. 3C

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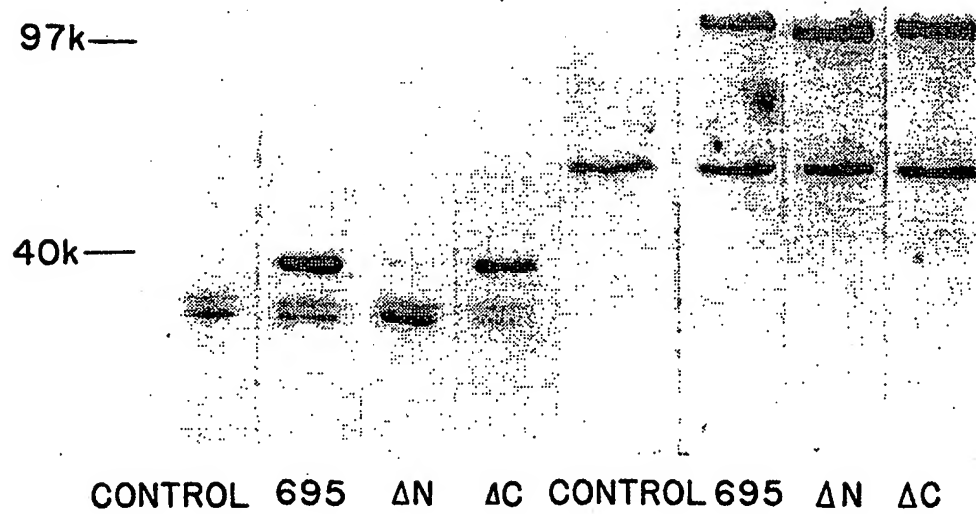


FIG. 3D

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TGTGGCAGGG AAGGGGCCAC C ATG GGA TGT ACG CTG AGC GCA GAG GAG AGA 51
 Met Gly Cys Thr Leu Ser Ala Glu Glu Arg 10
 1 5

GCC GCC CTC GAG CGG AGC AAG GCG ATT GAG AAA AAC CTA AAA GAA GAT 99
 Ala Ala Leu Glu Arg Ser Lys Ala Ile Glu Lys Asn Leu Lys Glu Asp 25
 15 20

GGC ATC AGC GCC GCC AAA GAC GTG AAA TTA CTC CTG CTG GGG GCT GGA 147
 Gly Ile Ser Ala Ala Lys Asp Val Lys Leu Leu Leu Gly Ala Gly 40
 30 35

GAA TCA GGA AAA AGC ACC ATT GTG AAG CAG ATG AAG ATC ATC CAT GAA 195
 Glu Ser Gly Lys Ser Thr Ile Val Lys Gln Met Lys Ile Ile His Glu 55
 45 50

GAT GGC TTC TCT GGG GAA GAC GTG AAG CAG TAC AAG CCT GTG GTC TAC 243
 Asp Gly Phe Ser Gly Glu Asp Val Lys Gln Tyr Lys Pro Val Val Tyr 70
 60 65

AGC AAC ACC ATC CAG TCT CTG CCG GCC ATT GTC CGG GCC ATG GAC ACT 291
 Ser Asn Thr Ile Gln Ser Leu Ala Ala Ile Val Arg Ala Met Asp Thr 85
 75 80

TTG GGC GTG GAG TAT GGT GAC AAG GAG AGG AAG ACG GAC TCC AAG ATG 339
 Leu Gly Val Glu Tyr Gly Asp Lys Glu Arg Lys Thr Asp Ser Lys Met 105
 95 100

GTG TGT GAC GTG AGT CGT ATG GAA GAC ACT GAA CCG TTC TCT GCA 387
 Val Cys Asp Val Val Ser Arg Met Glu Asp Thr Glu Pro Phe Ser Ala 115
 110 120

FIG. 4A-1

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GAA CTT CTT TCT GCC ATG ATG CGA CTC TGG GGC GAC TCG GGG ATC CAG	435
Glu Leu Ser Ala Met Met Arg Leu Trp Gly Asp Ser Gly Ile Gln	
125 130 135	
GAG TGC TTC AAC CGA TCT CGG GAG TAT CAG CTC AAT GAC TCT GCC AAA	483
Glu Cys Phe Asn Arg Ser 145	
140	
TAC TAC CTG GAC AGC CTG GAT CGG ATT GGA GCC GGT GAC TAC CAG CCC	531
Tyr Tyr Leu Asp Ser Leu Asp Arg Ile Gly Ala Gly Asp Tyr Gln Pro	
155 160 165 170	
ACT GAG CAG GAC ATC CTC CGA ACC AGA GTC AAA ACA ACT GGC ATC GTA	579
Thr Glu Gln Asp Ile Leu Arg Thr Arg Val Lys Thr Thr Gly Ile Val	
175 180 185	
GAA ACC CAC TTC ACC TTC AAG AAC CTC CAC TTC AGG CTG TTT GAC GTC	627
Glu Thr His Phe Thr Phe Lys Asn Leu His Phe Arg Leu Phe Asp Val	
190 195 200	
GGG GGC CAG CGA TCT GAA CGC AAG AAG TGG ATC CAC TGC TTT GAG GAT	675
Gly Gly Gln Arg Ser Glu Arg Lys Lys Trp Ile His Cys Phe Glu Asp	
205 210 215	
GTC ACG GCC ATC ATC TTC TGT GTC GCA CTC AGC GGC TAT GAC CAG GTG	723
Val Thr Ala Ile Ile Phe Cys Val Ala Leu Ser Gly Tyr Asp Gln Val	
220 225 230	
CTC CAC GAG GAC GAA ACC ACG AAC CGC ATG CAC GAG TCT CTC ATG CTC	771
Leu His Glu Asp Glu Thr Thr Asn Arg Met His Glu Ser Leu Met Leu	
235 240 245 250	

FIG. 4A-2

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TTC GAC TCC ATC TGT AAC AAC AAG TTT TTC ATT GAT ACC ACC TCC ATC ATC ATC	819
Phe Asp Ser Ile Cys Asn Asn Lys Phe Phe Ile Asp Thr Ser Ile Ile	265
CTC TTC CTC AAC AAG AAC GAC CTC TTT GGC GAG AAG ATT AAG AAG TCA	867
Leu Phe Leu Asn Lys Lys Asp Leu Phe Phe Gly Glu Lys Ile Lys Lys Ser	280
CCC TTG ACC ATC TGC TTT CCC GAA TAC CCA GGC TCC AAC ACC TAT GAA	915
Pro Leu Thr Ile Cys Phe Pro Glu Tyr Pro Gly Ser Asn Thr Tyr Glu	295
GAT GCA GCT GCC TAC ATC CAA ACA CAG TTT GAA AGC AAA AAC CGC TCA	963
Asp Ala Ala Ala Tyr Ile Glu Thr Gln Phe Glu Ser Lys Asn Arg Ser	310
CCC AAC AAA GAA ATT TAC TGT CAC ATG ACT TGT GCC ACA GAC ACG AAT	1011
Pro Asn Lys Glu Ile Tyr Cys His Met Thr Cys Ala Thr Asp Thr Asn	330
AAT ATC CAG GTG GTA TTC GAC GCC GTC ACC GAC ATC ATC ATT GCC AAC	1059
Asn Ile Gln Val Val Phe Asp Ala Val Thr Asp Ile Ile Ala Asn	345
AAT CTC CCG GCC TGC GGC TTG TAC TGACCTCTTG TCCTGTATAG CAACCTATT	1113
Asn Leu Arg Gly Cys Gly Leu Tyr	350

FIG. 4A-3

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GA CTGCTTCA TGGACTCTTT GCTGTTGATG TTGATCTCCT GGTAGCATGA CCTTTGGCCT 1173
TTGTAAGACA CACAGCCTTT CTGTACCAAG CCCCTGTCTA ACCTACGACC CCAGAGTGAC 1233
TGACGGCTGT GTATTCTCTG AGAATGCTGT AGAATACAGT TTTAGTTGAG TCCTTACATT 1293
TAGAACTTGA AAGGATTTTA AAAACAATAA CAAAACCAT TTCTCATGTG CTTTGTAGCT 1353
TTAAAAGAAA AAAGGAACAC TCACCATTTA ATCCATATTT CCTTTTATT TTGAAGTTTA 1413
AAAAAAAAT GTCTGTACCC ACACCCCTCC CCTTCCCAC CTCAGCAGAA CTGGGGCTGG 1473
CACACAGAGG CAGTGTCTGG CCTGGCCCT CCTAGGGCTT CTGTGCAGCC CATGGCTGGT 1533
GGGAACATGT CAGGCTAGTC TGTCTAGAAG GCCACTGGCC ACTGTACCCA CCTTCCCA 1593
TGCCTGTGGG CTGCCAGAC ACCTCATATA CCACCAGGCA GTGGCAGCTC CGCCCTGCTC 1653
AGCCATGCGA CTCCAAACAC ACTCAAAGTT TGGGTAGAAA AAGCACAGCT CTGGCAGGGG 1713
TAGCTGCCAC AGACAAACGT CATCACCTAT AGAAATCCAG CCTATAGAA GCAATTCACC 1773
CAGCCCTTC CTACACTCC TTTGTGTGT TAACTTTTGT GTTTTCTGG TCCTAGTGAG 1833
TGCCTCCCAT GCATACCTGA CCAGCTCTGC CAGTGTCTGG GGTCTGGGA ACAGGGGTG 1893
TGTGCTTGG TTTTGG 1910

FIG. 4A-4

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GCTGTGGCAG GGAAGGGGCC ACC ATG GGA TGT ACG CTG AGC GCA GAG GAG 50
 Met Gly Cys Thr Leu Ser Ala Glu Glu
 1 5
 AGA GCC GCC CTC GAG CCG AGC AAG GCG ATT GAG AAA AAC CTC AAA GAA 98
 Arg Ala Ala Leu Glu Arg Ser Lys Ala Ile Glu Lys Asn Leu Lys Glu
 10 15 20 25
 GAT GCC ATC AGC GCC AAA GAC GTG AAA TTA CTC CTG GGG GCT 146
 Asp Gly Ile Ser Ala Ala Lys Asp Val Lys Leu Leu Leu Gly Ala
 30 35 40
 GGA GAA TCA CGA AAA AGC ACC ATT GTG AAG CAG ATG AAG ATC ATC CAT 194
 Gly Glu Ser Gly Lys Ser Thr Ile Val Lys Gln Met Lys Ile Ile His
 45 50 55
 GAA GAT GGC TTC TCT GGG GAA GAC GTG AAG CAG TAC AAG CCT GTG GTC 242
 Glu Asp Gly Phe Ser Ser Gly Glu Asp Val Lys Gln Tyr Lys Pro Val Val
 60 65 70
 TAC AGC AAC ACC ATC CAG TCT CTG CCG GCC ATT GTC CCG GCC ATG GAC 290
 Tyr Ser Asn Thr Ile Gln Ser Leu Ala Ala Ile Val Arg Ala Met Asp
 75 80 85
 ACT TTG GGC GTG GAG TAT GGT GAC AAG GAG AGG AAG ACG GAC TCC AAG 338
 Thr Leu Gly Val Glu Tyr Gly Asp Lys Glu Arg Lys Thr Asp Ser Lys
 90 95 100 105
 ATG GTG TGT GAC GTG GTG AGT CGT ATG GAA GAC ACT GAA CCG TTC TCT 386
 Met Val Cys Asp Val Val Ser Arg Met Glu Asp Thr Glu Pro Phe Ser
 110 115 120

FIG. 4B-1

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434 GCA GAA CTT CTT TCT GCC ATG ATG CGA CTC TGG GGC GAC TCG GGG ATC
 Ala Glu Leu Leu Ser Ala Met Met Arg Leu Trp Gly Asp Ser Gly Ile
 125 130 135
 482 CAG GAG TGC TTC AAC CGA TCT CGG GAG TAT CAG CTC AAT GAC TCT GCC
 Gln Glu Cys Phe Asn Arg Ser Arg Glu Tyr Gln Leu Asn Asp Ser Ala
 140 145 150
 530 AAA TAC TAC CTG GAC AGC CTG GAT CGG ATT GGA GCC GGT GAC TAC CAG
 Lys Tyr Tyr Leu Asp Ser Leu Asp Arg Ile Gly Ala Gly Asp Tyr Gln
 155 160 165
 578 CCC ACT GAG CAG GAC ATC CTC CGA ACC AGA GTC AAA ACA ACT GGC ATC
 Pro Thr Glu Gln Asp Ile Leu Arg Thr Arg Val Lys Thr Thr Gly Ile
 170 175 180 185
 626 GTA GAA ACC CAC TTC ACC TTC AAG AAC CTC CAC TTC AGG CTG TTT GAC
 Val Glu Thr His Phe Thr Phe Lys Asn Leu His Phe Arg Leu Phe Asp
 190 195 200
 674 GTC GGG GGC CAG CGA TCT GAA CGC AAG AAG TGG ATC CAC TGC TTT GAG
 Val Gly Gly Gln Arg Ser Glu Arg Lys Lys Trp Ile His Cys Phe Glu
 205 210 215
 722 GAT GTC ACG GCC ATC ATC TTC TGT GTC GCA CTC AGC GGC TAT GAC CAG
 Asp Val Thr Ala Ile Ile Phe Cys Val Ala Leu Ser Gly Tyr Asp Gln
 220 225 230
 770 GTG CTC CAC GAG GAC GAA ACC ACG AAC CGC ATG CAC GAA TCC CTG AAG
 Val Leu His Glu Asp Glu Thr Thr Asn Arg Met His Glu Ser Leu Lys
 235 240 245

FIG. 4B-2

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CTC TTC GAC AGC ATC TGC AAC AAC AAG TGG TTC ACA GAC ACA TCT ATT      818
Leu Phe Asp Ser Ile Cys Asn Asn Lys Trp Phe Thr Asp Thr Ser Ile
250 255 260

ATC CTG TTT CTC AAC AAG AAG GAC ATA TTT GAG GAG AAG ATC AAG AAG      866
Ile Leu Phe Leu Asn Lys Lys Asp Ile Phe Glu Glu Lys Ile Lys Lys
270 275 280

TCC CCA CTC ACC ATC TGC TTT CCT GAA TAC ACA CGC CCC AGT GCC TTC      914
Ser Pro Leu Thr Ile Cys Phe Pro Glu Tyr Thr Gly Pro Ser Ala Phe
285 290 295

ACA GAA GCT GTG GCT CAC ATC CAA GGG CAG TAT GAG AGT AAG AAT AAG      962
Thr Glu Ala Val Ala His Ile Gln Gly Gln Tyr Glu Ser Lys Asn Lys
300 305 310

TCA GCT CAC AAG GAA GTC TAC AGC CAT GTC ACC TGT GCC ACG GAC ACC      1010
Ser Ala His Lys Glu Val Tyr Ser His Val Thr Cys Ala Thr Asp Thr
315 320 325

AAC AAC ATC CAA TTC GTC TTT GAT GCC GTG ACA GAT GTC ATC ATC GCC      1058
Asn Asn Ile Gln Phe Val Phe Asp Ala Val Thr Asp Val Ile Ile Ala
330 335 340 345

AAA AAC CTA CGG GGC TGT GGA CTC TAC TGAGCCCTGG CCTCCTACCC      1105
Lys Asn Leu Arg Gly Cys Gly Leu Tyr

AGCCTGCCAC TCACTCCTCC CCTGGACCCA GAGCTCTGTC ACTGCTCAGA TGCCCTGTTA 1165
ACTGAAGAAA ACCTGGAGGC TAGCCTTGGG GGCAGGAGGA GGCATCCTTT GAGCATCCCC 1225
ACCCACACCA ACTTCAGCCT CGTGACACGT GGGACACAGG TTGGGCAGAG GTGTGGAACA 1285

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FIG. 4B-3

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GCACAAGGCC AGAGACCACG GCATGCCACT TGGGTGCTGC TCACTGGTCA GCTGTGTGTC 1345
TTACACAGAG GCGAGTGGG CAACACTGCC ATCTGATTCA GAATGGGCAT GCCCTGTCTT 1405
CTGTACCTCT TGTTCAGTGT CCTGGTTTCT CTTCACCTT GGTGATAGGA TGGCTGGCAG 1465
GAAGGCCCCA TGGAAGGTGC TGCTTGATTA GGGGATAGTC GATGGCATCT CTCAGCAGTC 1525
CTCAGGGTCT GTTTGGTAGA GGGTGGTTTC GTCGACAAAA GCCAACATGG AATCAGGCCA 1585
CTTTTGGGGC GCAAAGACTC AGACTTTGGG GACGGGTTCC CTCCTCCTTC ACTTTGGATC 1645
TTGGCCCCCTC TCTGGTCATC TTCCCTTGCC CTGCGGCTCC CCAGGATACT CAGCCCTGAC 1705
TCCCATGGGG TTGGGAATAT TCCTTAAGAC TGGCTGACTG CAAAGGTCAC CGATGGAGAA 1765
ACATCCCCTGT GCTACAGAAAT TGGGGGTGGG ACAGCTGAGG GGGCAGGCGG CTCCTTCCCTG 1825
ATAGTTGATG ACAAGCCCTG AGAATGCCAT CTGCTGGCTC CACTCACACG GGCTCAACTG 1885
TCCTGGGTGA TAGTGACTTG CCAGGCCACA GGCTGCAGGT CACAGACAGA GCAGGCAAGC 1945
AGCCTTGCAA CTGCAGATTA CTTAGGGAGA AGCATCCTAG CCCAGCTAA CTTTGGACAG 2005
TCAGCATATG TCCCTGCCAT CCTAGACAT CTCCAGTCAG CTGGTATCAC AGCCAGTGGT 2065
TCAGACAGGT TTGAATGCTC ATGTGGCAGG GGGCCCGGTA CCCAGCTTTT GTTCCCTTTA 2125
GTGAGGGTTA ATTGGCGCT TGGGCTAATC ATGGTCATAG CTGTTGGGCG TTGCTGGCGT 2185
TTTTCCATAG GCTCCGCCCC CTGACGAGAT CACAAAATC GACGCTCAAG TCAGAGGTGG 2245
CGAAACCGAC AGACTATAAG ATACCAGGC 2274

FIG. 4B-4

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/01712**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(5) : G01N 33/543; C12Q 1/68; C07K 15/00

US CL : 436/518; 435/6; 530/350

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 436/518, 536; 435/6, 7.2, 7.21; 530/350

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, Dialog

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X, P	Nature, Vol. 362, issued 04 March 1993, Nishimoto et al., "Alzheimer amyloid protein precursor complexes with brain GTP-binding protein Go," pages 75-79, see entire document.	1-20, 27-29

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	* T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
* A document defining the general state of the art which is not considered to be part of particular relevance	* X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
* E earlier document published on or after the international filing date	* Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
* L documents which may throw doubt on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	* G document member of the same patent family
* O documents referring to an oral disclosure, use, exhibition or other means	
* P documents published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

18 APRIL 1994

Date of mailing of the international search report

25 APR 1994

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized officer

DONNA C. WORTMAN

Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/01712

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

- I. Claims 1-20, 27-29, drawn to a composition and a method of use, Class 436, Subclass 518, and Class 530, subclass 350.
- II. Claims 21-26, drawn to a treatment method, Class 512, Subclass 12.

Groups I and II do not share a common special technical feature as represented in PCT Rule 13.2 because they are drawn to completely different methods requiring different process steps for completion. Note that PCT Rule 13.2 does not provide for multiple methods within a single inventive concept.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-20, 27-29

Remark on Protest

☐

The additional search fees were accompanied by the applicant's protest.

☐

No protest accompanied the payment of additional search fees.